CONTRACT NO.: DAMD17-88-C-8033

TITLE: Social Behavior, Prolactin and the Immune Response

PRINCIPAL INVESTIGATOR: Bradford N. Bunnell

CO-PRINCIPAL INVESTIGATOR: W. Ben Iturrian

PERFORMING ORGANIZATION: Department of Psychology
University of Georgia Research
Foundation, Inc.
Graduate Studies Research Center
Athens, GA 30602

REPORT DATE: April 1, 1989

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
FORT DETRICK
FREDERICK, MARYLAND 21701-5012

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: University of Georgia Research Foundation, Inc. — 6b. OFFICE SYMBOL (If applicable): NA	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Graduate Studies Research Center, University of Georgia, Athens, GA 30602	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: US Army Medical Research and Devel. Command — 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: Contract No. DAMD17-88-C-8033
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
62787A	3E1-62787A879	AA	013

11. TITLE (Include Security Classification)
Social Behavior, Prolactin and the Immune Response

12. PERSONAL AUTHOR(S)
B. N. Bunnell and W. B. Iturrian

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 10/30/87 TO 10/29/88	1989, April, 1	97

16. SUPPLEMENTARY NOTATION

17. COSATI CODES

FIELD	GROUP	SUB-GROUP
05	08	
05	05	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Immune system; nonhuman primates; social behavior; stress; Prolactin; cortisol; ACTH; mitogens, ConA; PHA
RA 3

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The goal of the project was to develop a nonhuman primate model for studying the effects of social stress on immune system function. In this pilot project, stress was defined as activation of the pituitary-adrenal axis. Immune system indicants were PBL blast responses to mitogen (ConA and PHA) stimulation. Particular interest was in seeking correlations between social variables, immune function indicants, and serum levels of prolactin as a presumptive immunofacilitory hormone. Mitogen responses to a physical stressor (footshock) were used for assay development and as a base for examining social stress effects. Social stress was manipulated by changing the composition of the social groups. Complex effects relating mitogen responses to social variables were found, with social manipulations producing suppression of PBL proliferation followed by a rebound in some situations; facilitation in others, depending upon the situation and the type of individual involved. Evidence indicated that increased social status associated with increased PBL response. Report includes descriptions of comparisons of thymidine and colorimetric assay techniques for responses to mitogen stimulation. (JS)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT: ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Mary Frances Bostian	22b. TELEPHONE (Include Area Code): 301-663-7325 — 22c. OFFICE SYMBOL: SGRD-RMI-S

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

_______ Where copyrighted material is quoted, permission has been obtained to use such material.

_______ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

_______ Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

X In conducting research using animals, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

_______ For the protection of human subjects, the investigators have adhered to policies of applicable Federal Law 45CFR46.

_______ In conducting research utilizing recombinant DNA technology, the investigators adhered to current guidelines promulgated by the National Institutes of Health.

3/15/89

PI Signature Date

Table of Contents

Introduction

This report describes the work conducted during a one year exploratory project involving the development of a nonhuman primate model for studying the effects of social stress on functions of the immune system. The specific technical objectives were:

1. To develop a nonhuman primate model for examining the effects of social stress on neuroendocrine modulation of immune function.

2. To use this model to examine the relationship between stress induced alterations in levels of the hormone prolactin (PRL) and cellular immune functions.

3. To develop experimental hypotheses about the ways in which social variables might affect the reciciprocal relationships between the neuroendocrine and immmune systems involved in the modulaton of an organism's response to stress.

A. General considerations. The idea that immune system function can be altered by psychosocial factors is not new, but we are only just beginning to understand the mechanisms by which this can occur. If psychosocial factors affect susceptibility to disease, the course of disease, or indeed whether or not a disease state even obtains, then there must be some way for such factors to influence the responses of the immune system. Recent evidence indicates that the neuroendocrine and immune systems interact and are capable of influencing the functions of each other. Of particular interest are the findings which indicate that the neuroendocrine mechanisms involved in an organism's response to stress can cause immunosuppression or immunostimulation.

The elucidation of the mechanisms involved in immunoregulaton are of central concern to contemporary immunology. Research during the past 20 years has demonstrated that the immune system has a complex intrinsic network of feedback mechanisms which confer upon it a remarkable capacity for autoregulation. More recent evidence has shown that such autonomy is limited, at least in part, by the effects of certain hormones and neurotransmitters on the immune processes. (Locke, Ader, Besedovsky, Hall, Solomon & Strom, 1985). The term *psychoneuroimmunology* has been coined by researchers to describe the field which studies the intimate and complex interrelationships between the immune, endocrine and central nervous systems. Data are available which demonstrate that experimental manipulations of hormone levels can either augment or depress immune responses and similar findings have been obtained from work with lesions and stimulation of the central

nervous system. This opens the way for studies of the ways in which environmental stimuli, perceptions, ideas, expectations, and emotions may influence the health and behavior of organisms by modifying the activities of the immune system (Cunningham, 1981). Such studies are relevant, not only to traditional interests in "psychosomatic" illness, but for more holisitic definitions of health and disease as well.

At least under some circumstances, psychosocial factors that have been characterized as stressors have been shown to alter immune responses (c.f., Plaut and Friedman, 1981). The objective of the series of experiments reported here was to examine the effects of manipulating a particular class of such psychosocial factors, namely the social environment of the animals, on immunomodulation in nonhuman primates. Sociality is an ubiquitous characteristic of primate societies, including man's. While social behavior and organization confer definite advantages on primate species, they can also be a potent source of stress.

Although there is still considerable uncertainty about the specific mechanisms which interrelate neural, endocrine, and immune system activities, a large number of putative chemomodulators have been identified and a variety of models for such mechanisms have been proposed. (See the review by Tecoma and Huey, 1985 and books by Guillemin, Cohen and Melnechuk, 1985; Berczi, 1986, and Plotnikoff, Faith, Murgo and Good, 1986. Other recent reviews include Spector, 1983; Schindler, 1985 and Jankovic', 1985.) A compilation of over 1000 publications on "mind and immunity" covering a five year period (Locke and Hornig-Rohan, 1983) and a collection of seminal papers on psychoneuroimmunology (Locke, et al, 1985) serve as a useful guide to the literature in this area. In sum, the reviews of the experiments on neuroendocrine/immune modulation suggest that the CNS and endocrine systems can exert a direct influence on cellular and humoral immune responses as well as many related host-defense mechanisms. Although there is a great deal of contradictory literature, it is generally believed that:

1.) The CNS, endocrine and immune systems are inextricably linked by numerous feedback loops.

2.) Two-way communication is carried out by both circulatory hormones and direct neural innervation.

3.) Molecular links among the CNS, endocrine and immune systems occur via membrane receptor sites on various cells of the immune system for neurotransmitters, hormones, and other neuroactive peptides. Likewise, receptors are present on the cells of the CNS and endocrine system for bioactive substances from the immune system, such as thymosins, lymphokines, etc.

4.) Several specific brain nuclei and neuroendocrine mechanisms, as well as the autonomic nervous system, all have a role in immune modulation.

In developing the present project, we were very interested in the potential role of the hormone prolactin (PRL) in neuroimmunomodulation. The demonstration of PRL receptors on both human and rat lymphocytes (Russell, Matrisian, Kibler, Larson, Poulos and Magun, 1984; Russell, Kibler, Matrisian, Larson, Poulos and Magun, 1985) has suggested yet another function be added to the more than 85 physiological actions (MacLeod, Thorner and Scapagnini, 1985) already established for PRL. Although much remains to be done before PRL can be established as a chemomodulator essential to the overall mediation of neuroendocrine/immune system interactions, an impressive variety of recent evidence implicates PRL as a physiological hormone in the regulation of immunocompetence. (See reviews by Nagy and Berczi, 1986; Russell, et al, 1985; Spangelo, Hall and Goldstein, 1985, and Bernton, Hartmann, Gilbreath, Holaday and Meltzer, 1986.) Levels of plasma prolactin provide a sensitive indicator of acute stress and habituate over repeated presentations of a stressor (e.g., Kant, Bunnell, Mougey, Pennington and Meyerhoff 1983). For several years, we have used plasma prolactin in our studies of social stress in nonhuman primates (Bunnell and Iturrian, 1988).

B. Stress, Immunity, and the Social Milieu. Attempts to give the concept of stress the status of an intervening variable or hypothetical construct have led to a variety of definitions in terms of situational factors, constellations of responses, and disturbances of homeostasis. For our purposes, stress will be defined simply as increased activity in the hypothalamic/ pituitary/adrenal axis and the term stressor will be defined as any variable which produces, or is capable of producing, such an increase in neuroendocrine activity. While we recognize some of the problems inherent in such a definition, it provides an adequate framework within which to view the data and ideas which follow.

There is an extensive literature documenting the effects of stress and stressors on immunity (see the reviews by Solomon and Amkraut, 1981; Monjan, 1981, Riley, 1981; Tecoma and Huey, 1985; Stein, 1985; Murgo, Faith and Plotnikoff, 1986; Soloman, 1987; Glaser, Rice, Sheridan, Fertel, Stout, Speicher, Pinsky, Kotur, Post, Beck and Kiecolt-Glaser, 1987, Jancovic', Markovic' and Spector, 1987 and Perez-Polo, Bulloch and Angeletti, 1987). Studies relating alterations in immunity to social variables are particularly intriguing. Among the early work in this area are papers indicating that members of stabilized social groups were more resistant to virus induced tumors and to encephalomyocarditis (Friedman, Glasgow and Ader,

1969) while stressful crowding - which involved both physical and social variables - increased susceptibility to malaria (Plaut, Friedman and Grota, 1971) and resulted in significantly lower antibody levels in response to immunization (Solomon, Levine and Kraft, 1968).

Differential housing of mice - either individually or in groups of five - selectively modified immune competency by reducing T helper, but not B cell, IL-1 or IL-2 production (Rabin, Lyte, Epstein and Caggiula, 1987). The results emphasized that some physiological manipulations have different effects on different levels of immune function. In another study, grouped mice had lower titers of circulating antibody and heavier adrenals than isolated mice; interestingly, the socially dominant animals in these groups had significantly higher antibody titers than the others (Vessey, 1964). Social hierarchy has also been related to susceptibility to the lethality of viral infection (Gross, 1972). Control rats, housed in the same room with stressed rats have been reported to be immunosuppressed (Steplewski and Vogel, 1986). Separation of mother and infant (Laudenslager, Reite and Harbeck 1982), hand rearing (Laudenslager, Held and Boccia, 1988), as well as isolation from peers (Reite, Harback and Hoffman, 1981) impaired cellular measures of immune function in primates. Immunosuppression may also be related to the increased mortality observed after bereavement (Schleifer, Keller, Camarino, Thornton and Stein, 1983).

Current literature emphasizes the complex bidirectional interactions of stress, endocrine, and psychosocial factors. Most investigators agree that a psychological stressor itself can alter immune function. (See reviews by Spector, 1987; Solomon, 1987, and Ader, Grota and Cohen, 1987.) For example, the immunosuppressive (Lysle, Cunnick, Fowler and Rabin, 1988) and neuroendocrine stress responses (Bunnell, Meyerhoff and Kant, 1988) produced by electric shock can be conditioned to environmental stimuli such that the presentation of such stimuli in the absence of shock elicits fear which can mediate the suppression of immune function and changes in pituitary and adrenocortical secretions. It also appears that most behavioral manipulations that produce analgesia (either opioid or nonopioid), or that induce fear or anxiety, suppress immune function. Also, a variety of drugs which induce anxiety suppress many immunological indices. It should be noted that a benzodiazepine antagonist and naloxone given in the absence of stress produce different alterations in the immune system than when they are used with stress (Pericic', Manev, Boranic', Poljak-Blazi and Lakic', 1987; Murgo, et al, 1986, and Arora, Hanna, Paul and Skolnick, 1987).

A nonhuman primate model should be useful in advancing the understanding of social stress/immune system mechanisms.

Sociality is among the primary ecological specializations evolved in the order *Primates* (Kummer, 1971). Except for a few prosimians and, possibly, orangutans, all primates live in social groups. In the primates, including man, group living confers many advantages in terms of access to resources, reduction of overt aggression, mutual care, and the like. Sociality can also be stressful as when resources are limited and competition increases, authority is challenged or group norms violated, expectations are not met, sacrifices are required by the group, or external forces put extra pressure on the group structure. Aggression may increase, members may have to adapt to changing status and roles, and the organization of the group may be disrupted. Analogies to stressful human social interactions are easy to identify in nonhuman primate groups. Low ranking members of the social hierarchy may be stressed by the constant vigilance required to insure that they do not offend animals of higher status, while top ranked animals may have to be continuously alert against challenges from below. Intragroup aggressive conflicts may result from competition between individuals and these may lead to breakdowns in established relationships and to further increases in conflicts. Changes in the membership of the group due to the addition or loss of individuals can strain the existing relationships. Stress may also be produced when difficulty is encountered in employing the usual mechanisms that have been developed for producing reconciliation between individuals. Differences in individual animals' abilities to cope with social stressors can lead to differences in levels of stress and, presumably, to individual differences in immune system function.

Social stress can be experimentally induced in captive groups of nonhuman primates in a number of ways. Perhaps the easiest is through manipulation of group membership (Bunnell and Iturrian, 1988). Once the social rank structure of the group is known, removal, or removal followed by replacement, of key members of the social hierarchy can produce increases in agonistic behavior and changes of interanimal relationships which are accompanied by increases in levels of the plasma hormones, such as cortisol and prolactin, which we use as indicants of stress. Similar, but more intense effects may be produced by introducing strangers to the group. Competition for food can induce stress and alter indices of immunocompetence (Laudenslager, et al, 1988). We have been developing a procedure which requires group living monkeys to work for their daily food rations as a model for studying competitive and cooperative modes of coping (or failing to cope) with limited resources. Changes in cortisol and ACTH levels have been used to identify and define social stress; correlations were sought between changes in the pattern of PRL secretion and indicants of immune system function.

C. Neuroendocrine Modulation of Immunity: Prolactin. Many changes occur in the chemistry of the organism during stress and it is often difficult to establish the true relationships between such changes and alterations in immune system response. The stimulation or inhibition of immune function are commonly reported, but there can also be a biphasic response during chronic stress or during recovery (Tecoma and Huey, 1985). Some components of the immune system are selectively changed during and after stress and sometimes changes opposite to those observed during stress persist for weeks or months after the stressor is withdrawn (Steplewski and Vogel, 1986). Little is known as to whether or not such persistent changes can also be found in the nervous and endocrine systems.

The neuroendocrine-adrenal axis modulaton of the immune system has been reviewed (Cooper, 1984; Hall, McGillis, Spangelo, Healy, Chrousos, Schulte and Goldstein, 1985, and Smith and Blalock, 1986). The adrenal hormones are capable of modulating the responsivity of all immunological cell types. Although ACTH and the glucocorticoids are historically correlated with immunosuppressive actions, they also potentiate antibody production and they are essential in maintaining lymphocytes in culture. Since glucocorticoids exert biphasic effects on immunity, the adrenal cortex should be considered "immunoregulatory" (Hall, et al, 1985).

As noted earlier, if the pituitary has an immunoregulatory function then some immune cell derived chemicals, immunohormones and immunotransmitters, must act as feedback signals capable of influencing the secretion of regulatory hormones from the pituitary. ACTH-like material derived from lymphocytes is one of the immunohormones. Other candidates include leukotrines, lymphokines, thymosin, histamine and various growth factors (Besedovsky, delRey and Sorkin, 1986; Berczi, 1986, Ch. 10).

The immune system may produce chemicals that influence the production of neuroendocrine hormones which directly alter host/immune responsiveness (Smith and Blalock, 1986). Although thymosin-1 was ineffective in stimulating steroidogenesis in monkeys, other lymphokine containing preparations are active in man (Hall, et al, 1985). Macrophages also synthesize their pituitary counterparts, ACTH and endorphin. It appears that the immune system and the pituitary-adrenal axis interact by common ligands and receptors but many of the active substances from the immune tissue have not been adequately identified. ACTH-like materials derived from lymphocytes blocked the effects of PRL (Nagy and Berczi, 1986).

All of the pituitary hormones that have significant influences on the immune system, i.e., ACTH, growth hormone

(GH) and PRL, are also "stress hormones" (Berczi, 1986, Ch. 14). Furthermore, other molecules involved in the stress response, including endorphin, enkephalin and vasoactive intestinal polypeptide (VIP) also have been correlated with immune responses, suggesting that there is a regulatory loop between the nervous, endocrine, and immune systems involving neurotransmitters, neuromodulators, and hormones (Smith and Blalock, 1986). Indeed, a partial list of "receptors" on the lymphocyte membrane included over 40 neurotransmitters, neuropeptides and neuromodulators (see reviews by Jankovic' and Spector, 1986 and Hall and Goldstein, 1985). The immunohormones and immunotransmitters that provide the direct afferent channels to the neuroendocrine systems have been studied less extensively, but the data suggest that the thymus and immunocytes emit electrical and chemical signals which influence neuroendocrine mechanisms to modify the secretion of pituitary hormones (Berczi, 1986). The influence of neuroendocrine signals on intrinsic immunoregulatory processes has been the subject of two recent reviews (Besedovsky, et al, 1985, 1986) and a summary of the influence of thymic and other immunohormones on hypothalamic cells and the adrenals is available (Hall, et al, 1985).

There is considerable evidence which indicates that PRL is an immunomodulatory hormone that may be essential for the development and function of the immune system (Spangelo, et al, 1985; Nagy and Berczi, 1986, and Bernton, et al, 1986). Indeed, PRL may be the equivalent of GH in the development and maintenance of immune competence. Recent evidence shows that there are specific PRL receptors in nuclei that activate protein kinase C (PKC) (Buckley, Crowe and Russell, 1988). Murine splenocytes make and secrete a lymphocyte prolactin-like protein in response to concanavalin A (ConA). High affinity prolactin receptors in primates and rodents are blocked by cyclosporine. PRL reverses the immunnosuppressive effects of bromocriptine

Prolactin should be particularly useful for model testing because it produces a complete biphasic lymphoid response as well as triggering new RNA and protein synthesis. PRL's activity in mitogen assays depends on the specific lymphoid tissue and other test conditions, including initial cell concentration, mitogen level, and PRL dose. Whether the PRL immunoregulating action is facilitory or inhibitory appears to depend upon the concentration of PRL used. Pharmacological concentrations generally are reported as immunosuppressive while physiological levels have complex actions, but generally enhance both antibody and cell mediated immunity via immune cell gene expression. Although PRL is a commitogen in lymphocytes, rat thymus and spleen cells express high affinity receptors and are PRL dependent for growth (Russell, et al, 1985). In addition, PRL plays an important role in the homing

of IgA producing cells and increases IgA binding 25 fold. PRL enhances Con A mitogenesis from 1 mM to 1 uM, but only if initial cell density is optimal. At lower or higher cell density, cell proliferaton is inhibited. Lipopolysaccaride (LPS) and phytohemaglutinin (PHA) induced mitogenesis are insensitive to PRL, but high doses will inhibit PHA.

In the NB2 lymphoma cells that have PRL dependent mitogenesis, peripheral bendoziazepines markedly increased proliferation by binding at a high affinity site (Laird, Duerson, Buckley, Montgomery and Russell, 1987). The stimulatory effects occurred from 10^{-10}M to 10^{-7}M while 10^{-6}M inhibited PRL stimulated mitogenesis when PRL was at its optimal concentration (10 ng/ml). The recognition site for ligand binding was neither GABA nor chloride dependent so an interaction between PRL and BDZ receptor site must occur.

D. Experimental Program: In order to demonstrate the feasability of a nonhuman primate model for studying the effects of social stress on immune function, five experiments were proposed for the pilot project. These are described in general terms in this section. A sixth experiment was conducted at the end of the project to examine the time course of changes in immune function following repeated footshock stress and this study was done in conjunction with observations of social behavior. Minor modifications to the five studies proposed originally will be described in the sections on each experiment. Also, the progress of additional work, including performance testing and the development of competitive and cooperative testing procedures will be presented.

Experiment 1. The first experiment was designed to check the procedures to be employed in scheduling the blood draws and doing the assays of immune function. Six adult males were subjected to moderate levels of repeated stress, using footshock as the primary stressor, over time periods varying from one to three weeks. During this time, and for a period of several weeks following administration of the stressor, blood samples were obtained twice a week for assay for cortisol, ACTH, and prolactin and for the mitogen assays. The objective was to follow the time course of stress hormone and immune function changes using a stressor which we were sure would produce elevations of cortisol and PRL when administered acutely. The animals were housed in individual cages in the laboratory during the study and no concurrent social behavioral observations were made.

Experiments 2 and 3. While Experiment 1, above, was in progress, the monkeys from a small, all-male group were separated and placed in single cages for several months. After baseline hormone and immune function data were gathered, the group was reformed. Social data were obtained daily and

included the total frequency of social behavior of each monkey, aggressive and submissive behavior frequencies, and frequencies of affiliative behaviors as measures of conciliation and coping behavior, and rank in the social hierarchy. We also monitored performance on a mult RI-RI operant schedule as a further behavioral indicant of stress. Correlations between the behavioral, hormone, and immune function data were examined. To induce a more intense level of social stress in this group of monkeys, three "stranger" males from other animal groups were then introduced, one-by-one, at 1 to 3 week intervals. We monitored the behavioral, hormonal, and immune function measures throughout the introductions and for several weeks afterwards. This group later served in the sixth experiment, which was a followup to Experiment 1.

Experiments 4 and 5. These used one of the large (n=43) groups of monkeys containing adults, juveniles and infants of both sexes. In experiment four we monitored the social behavior of the entire troop and obtained hormone and immune function data from 11 monkeys (5 adult males, 2 adult females, 1 subadult male, 1 subadult female, and 1 juvenile male and 1 juvenile female) during normal social behavior. Experiment five involved the introduction of two stranger males into the troop with an interval of 1 month between the introductions. Four days after the second introduction, a young adult male who had been removed from the group several months before was reintroduced to the troop. The purpose of these manipulations was to cause disruption of the social group, to increase social stress, and to determine the effects of the manipulations on members of different age/sex classes within the troop. During the course of the studies, several troop members were removed for treatment of wounds or illness and then replaced. This provided information on the effects of moving familiar animals in and out of the social group.

Experimental Studies

A. Subjects and General Procedures

1. Monkey Colony:

The subjects used in the project were drawn from a colony of approximately 100 *Macaca fascicularis* monkeys. This colony had been used in an earlier project involving the development of a behavioral test battery to evaluate the effects of chemicals used as antidotes and prophylactics to CW agents on social behavior and performance on laboratory tasks (Bunnell and Iturrian, 1988). The monkeys were divided into four groups. Two of these, called "T-Troop" and "NT-Troop", were breeding groups containing all age and sex classes of animals. The other two groups were small, all-male troops. "I-Troop" initally consisted of six adult males that had been

together for several years prior to the beginning of the project. "C-Troop" contained six adult males which had been removed from NT-Troop in 1984 and housed in the laboratory. T-, NT- and I-Troops were housed together in large outdoor compounds. The C-Troop males were housed in individual cages in the laboratory. They were used in the first study on the effects of footshock stress on immune function and in the development of a laboratory task for inducing social competition and cooperation. Later, three members of C-Troop were used in testing the effects of introducing "strangers" to the other social groups. During the year, the experimental work on the effects of social variables on immune function was conducted with I-Troop and NT-Troop, and T-Troop served only in pilot testing. The composition of the various groups at the beginning of the project at the end of October, 1987 is given in Table 1:

Table 1

Group Composition as of 1 November 1987
(Number of monkeys in each age/sex category.)*

		Adult		Subadult		Juvenile		Infant	
		M	F	M	F	M	F	M	F
TROOP:									
"T"	N = 44:	7	16	4	3	6	2	3	3
"NT"	N = 43:	8	13	2	1	6	7	3	3
"I"	N = 6:	6	0						
"C"	N = 6:	6	0						
TOTAL	N = 99:	27	29	6	4	12	9	6	6

* Males (M) over 6 years old and females (F) over 4 years old are classified as adults. Males 4-6 and females 4 years old are subadults. Juveniles are over 1 year old (both sexes)(Angst, 1975).

The outdoor compounds used to house T-, NT-, and I-Troops were 14.1 m long, 3.1 m wide, and 2.0 m high. Each compound was equipped with perches, swings, and a water fountain and contained an observer station, 1.6 m square, in the center from which observations of social behavior were recorded. The compounds were connected to heated and airconditioned indoor quarters by runways 1.2 m in cross section. The runways were partially covered to provide shelter from rain and sun when the animals were outside. The indoor quarters consisted of cages 6.1 m long x 1.2 m wide x 2.2 m high which were equipped with water fountains and perches. Small guillotine doors on the

sides of these cages were used to collect the animals in transport boxes for testing in the laboratory. Guillotine doors between the indoor cages and the runways, and between the runways and the compounds, allowed the animals to be moved to different sections of the living quarters during social testing and daily cleaning.

The 6 males of C-Troop were housed in a battery of individual cages in a separate colony room in the laboratory. An adjacent suite contained a cage, measuring 1.8 m x 1.8 m x 1.8 m, in one room and an observer station, equipped with one way windows, in the other. The C-Troop monkeys were brought from their colony cages and placed in this cage for studies of competitive and cooperative social behavior.

Yet another room contained 18 individual cages and was used as a holding facility when animals were removed from their troops and during laboratory testing . Monkeys being tested were trained to enter transport cages from their group cages and were adapted to a restraint device used to hold the animals while blood was drawn for the hormone and immune function assays. As much as possible, the capture and restraint procedure was made a part of the daily routine for all animals undergoing experimental testing. Preexperimental blood draws for assays of serum cortisol and prolactin were used to monitor the individual monkey's adaptation to the capture and handling process.

2. Social Behavior:

a. Social Organization in *M. fascicularis*:

Sociality, defined as the tendency to associate with one's fellows and to form social groups, is characteristic of most primate species, including man. The ubiquity of primate societies makes the study of nonhuman primate groups of potential importance in understanding certain aspects of human social behavior and organization and offers the possibility of using this animal model as a tool for testing drug effects on social behavior. However, while most, if not all, nonhuman primate species evince only one of several possible social organizations, man is much more flexible in terms of the kinds of social organization exhibited in his societies. Thus, generalizations from studies of monkey social behavior must be made with caution; to gain the maximum benefit from such data, one must view the results in the context of the particular social organization exhibited by the species of primate being studied.

In *M. fascicularis*, there are two elements of the social organization of the monkey troops that are particularly important. The first of these is the social dominance hierarchy among the adult males, while the second consists of a social hierarchy of the matriarchies present in the group. In a matriarchy, an old female, her daughters, her daughters' daughters, etc. and their infant offspring form a social unit. Each matriarchical unit has a social rank within the hierarchy of matriarchies such that all members of a unit have the same social status as the matriarch and an increase or a drop in the social rank of the matriarch will be accompanied by a corresponding change in the status of the members of her matriarchy (Angst, 1975). The two kinds of hierarchies function to promote cohesion within the group. Once the dominance/subordination relationships are established, each animal knows his or her status with regard to very other animal in the group and overt aggression is greatly reduced. Maintenance of the social rank structure is accomplished by threats and submissive signals that do not involve physical contact and injuries are rare. In a stable social group, physical contacts generally involve mutual grooming, sitting with one another, hugging and embracing, sexual behavior, and other affiliative behaviors which promote group cohesion and appear to reduce tensions between individuals. The top-ranked, or "alpha" male plays a key role in controlling the activities of the other members of the social group (Wechsler, 1986).

Although one or more high ranking matriarchs may outrank some of the lower ranking adult males, the male dominance hierarchy and the hierarchy of matriarchies seem to function more or less independently within the group. Males tend to interact with other adult males in the hierarchy and with females on an individual basis during grooming and copulation. (There may be some more subtle relationships between male status and the matriarchical structure, however. On occasion we have observed that the loss of a matriarch, in addition to resulting in the loss of status of her matriarchy, has been accompanied by a loss of rank of her adult sons in the male hierarchy (Bunnell, 1982)).

Operationally, the social rank of an animal is defined in terms of defeats. The occurrence of a submissive behavior in a monkey indicates that the animal is inferior in rank to the animal toward which it directs the submissive signal. (Similarly, the animal is dominant over monkeys which direct submissive behaviors toward it.) The social rank hierarchy is constructed by noting the submissive member of all possible pairs of animals and combining this information to determine the relationships between each animal and all other members of the group. In captive groups of *M. fascicularis*, the hierarchy among adult males is usually linear, that is, all of the other males submit to the alpha male, the second-ranked

("beta") male submits to the alpha male, but is in turn submitted to by the remainder of the group, and so on. Occasionally, an alliance between two males will occur and together these two animals will often hold a higher rank in the structure than they would as individuals. Reversals in rank can also be present such that animal E submits to D, F submits to E, but D submits to F. These departures from linearity are usually seen among the lower ranking animals of the group. With respect to the matriarchies, dominance submission relationships tend to be more complicated. As noted above, the female offspring of the matriarch usually hold the same rank that she does with respect to nonmembers of her matriarchy. Within the matriarchy, however, a dominance hierarchy exists among the adult females and their juvenile offspring appear to have the same ranks as their mothers in the subgroup.

b. Social Data Collection:

Social behavior is scored using the behavior categories given in Table 2. The observers record the code for the animal exhibiting the behavior, a code for the behavior itself, and then a code for the animal that is the recipient of the behavior. The two procedures utilized in gathering data are the "group scan" and the "focal animal" techniques. In a group scan, the observer watches the entire group and records every behavior that occurs as it happens; a modified version of a group scan involves looking at each monkey in sequence and recording what it is doing at the instant it is scanned. The focal animal procedure involves attending to only one animal for a period of time and recording the direction and nature of all behavior it either does or receives during that time.

Table 2

M. fascicularis Behavior Categories

Agonistic Behaviors:		
	Aggressive	Chase
		Threat (open-mouth)
		Charge
		Slap
		Bite
	Submissive	Avoid
		Grimace
		Squeal
		Flee
	Other Agonistic	Lid
		Lip Smack
		Enlist
		Demonstrate
Sexual Behaviors:		
		Sexual Present
		Mount (no thrusting)
		Mount (with thrusting)
		Masturbate
		Genital Manipulation (other animal)
		Genital Sniff (other animal)
Affiliative Social Behaviors:		
		Present to Groom
		Groom
		Ventral-Ventral Hug
		Ventral-Dorsal Hug
		Sit-Next-To (Physical contact)
		Play (not included in analysis)
Non-Social Behaviors:		
		Self Groom
		Move
		Sit - No Social Interaction

In the analyses of the social data obtained by the scan techniques, a laboratory computer provided a listing of the frequencies of each behavior performed by each monkey and the frequencies with which it directed these behaviors to each of the other monkeys in the troop. These data were then used to produce a series of matrices describing the basic social organization and dynamics of the group. Usually, several days' data were combined in these analyses. In this procedure, the computer sorted all of the data and determined the social rank of each animal on the basis of who was defeated by whom, using the submissive behavior categories listed in Table 2. This defines the social dominance hierarchy for the troop. The computer then printed a series of six matrices in which the animals were listed in the order of their social rank. In each matrix, the frequency of occurrence of each behavior, or class of behaviors selected for inclusion in that matrix, was given for each animal with respect to every other animal in its troop. (We are limited to 24 x 24 matrices; in scoring the behavior in the larger troops, the behavior of the 23 oldest animals in each group was scored and the 24th slot was used to represent all the remaining infants and juveniles in the troop). Four of the six matrices were used to summarize the combinations of behaviors listed under the functional categories Aggressive, Submissive, Sexual, and Affiliative as given in Table 2. For the other two matrices, any individual behavior of interest could be selected. Thus, we might look at threat - a measure of noncontact aggression - in order to compare it with the matrix for overall aggression, or obtain separate matrices for grooming, which is included in the Other Social matrix and play, which is not. Examples of these matrices may be found in Appendix A.

The data from each focal animal observation were analyzed individually or summarized across observations to provide baseline information on response frequencies and directions to which the data from observations during experimental manipulations could be compared. In some instances, the matrix programs were used with the focal data by combining these data for several animals for one or more days. For other purposes, useful information was obtained by combining both scan and focal data in a single matrix analysis.

c. Experimental Manipulations of Social Behavior:

Social behavior within the groups was manipulated in three ways. In the first method, all of the I-Troop males were brought into the laboratory and housed individually for several months. The group was then reformed by putting individuals back into the social compound, one by one at 15 min intervals, until all four were back together. In the removal and replacement procedure, one or more monkeys were removed from a troop for

periods ranging from a few days to a month or more and then returned to their original group. Some removals and replacements were planned to fit the experimental protocols, others were unscheduled and involved the need to removal an animal for treatment of an illness or injury. We had used the removal and replacement procedure as the primary method for manipulating social behavior in the troops in our earlier work and found that the amount and nature of the changes in social behavior induced by the procedure were a function of the social rank of the animals removed and of the stability of the group structure at the time the manipulations occurred (Bunnell and Iturrian, 1988). The third procedure was to introduce strangers - adult males taken from one of the other troops - into the troop being studied. This produced large amounts of aggression that was directed toward the stranger. It also allowed for the study of the strangers' ability to cope with the situation. Coping was operationally defined in terms of increases in affiliative behaviors between the resident animmals and the stranger across the days following the introduction. Details about the use of the three methods and their effects on social behavior will be found in part C of this section of the report.

Yet another way of manipulating social behavior involved observing the animals from C-Troop in sets of from 2 to 6 monkeys while the animals were working for food on an operant schedule in a special laboratory social test cage. Since all but the top and bottom ranked monkeys in the troop are dominant to at least one and subordinate to at least one other animal, pair testing allowed the observation of a given monkey's performance both when he was the dominant and when he was the subordinate in the situation. This procedure was used in pilot studies used in developing tests of competition and cooperation which we hope to use in future work on stress and immune function.

3. Performance Testing:

We have found that performance on a variety of laboratory tasks is subject to disruption by social stress (Bunnell and Iturrian, 1988). During the year, three operant reinforcement paradigms have been employed to examine social stress/performance effects; one of these looked at individual performance following social manipulations; the other two have examined performance while the animals were together in pairs or larger groups.

A laboratory room containing six primate operant chambers was available for testing. Reinforcement schedules and data collection were controlled by a Digital Equipment Corporation PDP 11/73 computer running under SKED-11 software. The chambers were used in the footshock studies conducted with the C-Troop and I-Troop males as well as in testing performance of the

I-Troop males on a positive reinforcement schedule during social group manipulations. Operant testing was done in the mornings, after social testing and blood sampling and before the morning feeding, so that the monkeys were fasted overnight. Procedural details will be found in part C1 of this section of the report.

In previous work (Bunnell & Iturrian, 1988) we had installed an operant panel, complete with cue light, manipulanda, and a pellet feeder in the indoor social test cage and examined performance on fixed ratio (FR) reinforcement schedules with 2-6 animals present in the cage. The presence of the panel produced a significant increase in the frequency of social interactions among the monkeys and, since only one animal could have access to the panel at a time, we were able to identify both agonistic and cooperative episodes as the animals exchanged places at the panel. During the present project, we installed a second operant panel next to the first and trained the six C-Troop males to perform on both panels. Activation of one or both panels, simultaneously or successively, and the use of different schedule requirements on different panels were used to develop procedures for tests of competition and cooperation. Procedural details and preliminary results will be found in part D of this section of the report.

An operant panal was placed in the outdoor compound housing NT-Troop and activated during social testing at various times during the project. Procedural details and comments on the use of this task will be given in Part D of this section.

4. <u>Assays</u>:

a. Mitogen assays:

At present there is no consensus among pychoneuroimmunologists as to the most appropriate strategy to use when attempting to document immune system modulation by neuroendocrine factors (See symposium discussion, Guillemin, et al., 1985, 243-252). It would have been ideal to do all the mitogen, specific antigen, cytotoxicity, granulocyte function and interleukin assays that are now available, this was not feasible in terms of time, the amount of blood required, or the frequency with which blood must be drawn to adequately monitor the dynamics of the processes of physiological adaptation to stress. Although pe ripheral blood lymphocyte (PBL) blast responses to mitogen stimulation are very crude indicators of immune system function, we have used them in our initial work to establish that the manipulation of social varables in our monkey species will indeed produce changes in these indicants and as the basis for determining the direction of the subsequent work we have proposed for a continuation of the project.

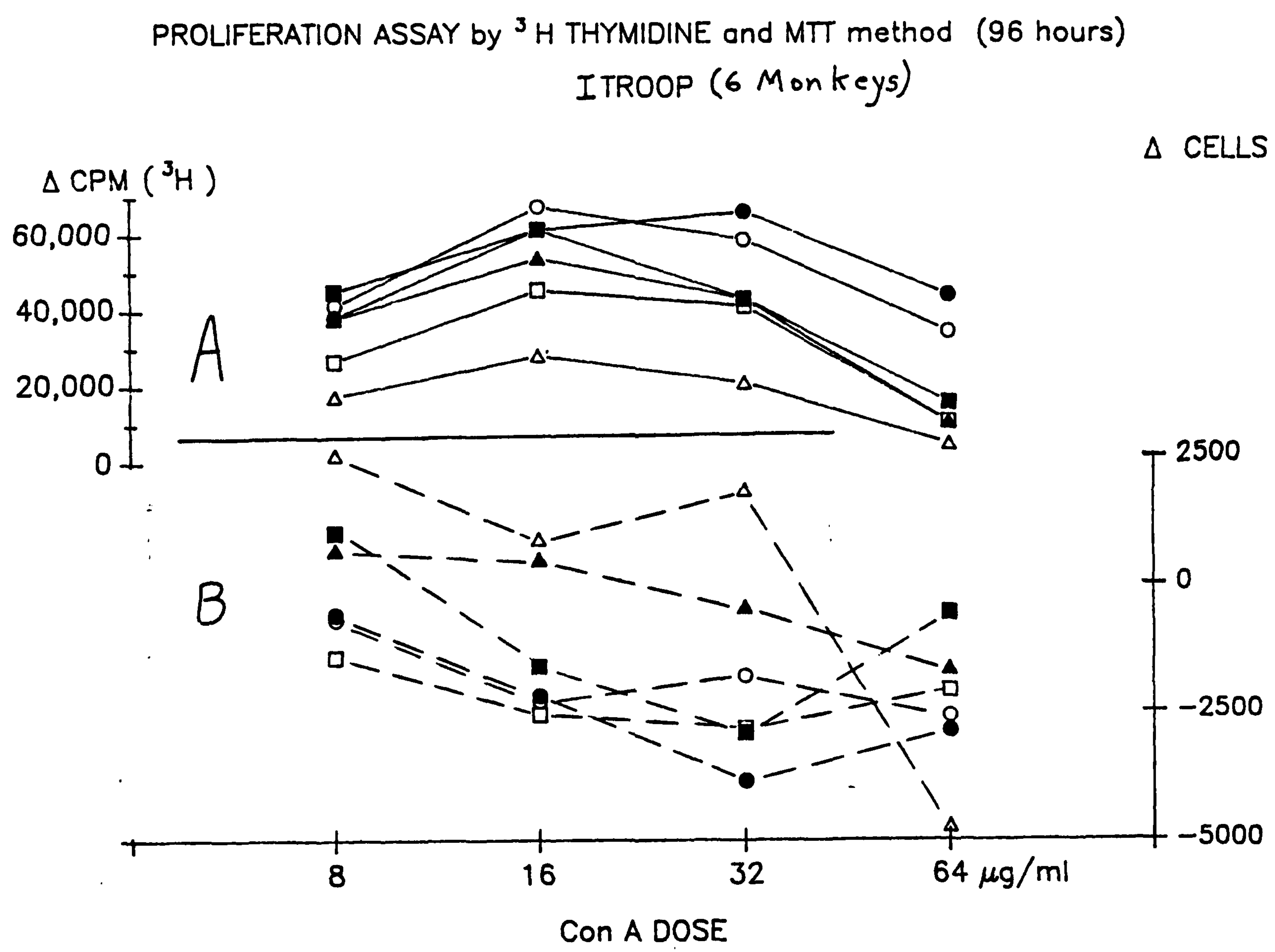


FIG. 1: COMPARISON OF THE ^{3}H THYMIDINE AND MTT METHODS

In our initial proposal, we indicated that we would use a colorimetric assay for this work. We were given to understand that the MTT assay (Chemicon Int. Corp.) developed by Mossman (1983) for determining cellular proliferation, cytotoxicity, growth and survival was an acceptable substitute for ^3H thymidine and cell harvesting. We encountered many problems in bringing the MTT assay on line and so we employed the ^3H thymidine assay for the initial experiments. We used the facilities of the Clinical Immunology Laboratory of the Unniversity's College of Veterinary Medicine to run assays on PBL responses to stimulation by Con A and PHA and to train our technician in the procedures.

After several false starts we adapted the MTT assay to use with our monkeys and were able to use it during the latter part of the year. During the last quarter of the project, we were able to set up to run the ^3H thymidine assays ourselves and made an effort to quantitate the differences between the two methods. We found a high correlation ($r = +.96$) between cell number and MTT optical density in unstimulated PBLs, but the correlation decreased as incubation time was increased following the addition of a mitogen. The optimal MTT stimulation index (SI) occurred after 48 hours of incubation. Although the optimal uptake of ^3H thymidine occurred after 96 hours of incubation (24 hr pulse with the ^3H label), the MTT assay demonstrated that MTT dye sensitive cells were rapidly dying (see Figure 1). It should be noted that the two assays were performed on aliquots from the same well of the tissue culture plate. Microscopic examination showed that the predominant cell type at 96 hr had a different morphology and only a small percentage of the cells took up the MTT dye.

A marked differences in individual monkey's PBL responses to mitogen stimuation was observed in both assays and for constant initial cell number as well. Although there was a tendency for high MTT SIs to be accompanied by lower SIs for ^3H thymidine in individual monkeys, the effect was not statistically significant within the group of six animals investigated. We suggest that the low thymidine SI results from a deficiency of a growth factor of an unknown nature, since mitogen responsiveness could be restored by adding 10% PBL from another monkey. Additional studies to document the phenomena would require the use of a cell sorter.

The PBL in our monkey species is very responsive to Con A and PHA stimulation, but it is a very fragile lymphocyte to culture. Although we have tried several different incubation mediums and growth factors, we have not been successful in in obtaining satisfactory mitogen responses from cultures of our monkey's lymphocytes after separating them from whole blood. We had proposed to do IL-2 assays in the purified system. However, Dr. Mark Laudenslager at the University of Colorado advised us

that he had spent several thousand dollars on IL-2 assay kits, but had been unsuccessful in inducing any changes by social manipulations in his bonnet macaques (Personal communication, FASEB Summer Conference on Neuroimmunomodulation, Copper Mountain, CO, Summer, 1988.) In consequence, we have not attempted any IL-2 assays during the past year; however, an expanded battery of assays has been incorporated into the proposal for continuing the work and this includes an examination of IL-2 production following mitogen, antigen, and anti-T3 stimulation to document lymphocyte functional status.

b. Hormone assays:

Serum PRL and cortisol were assayed in duplicate using kits obtained from Cambridge Medical Diagnostics, Inc. In our hands, these assays yield a recovery of between 92-96% for cortisol and 98-110% for PRL. Intraassay and interassay coefficients of variation for cortisol are 4% and 7.5% respectively; equivalent values for PRL are 8.5% and 11.1%. Sensitivity of the cortisol assay is 1 ug/100ml; we have modified the prolactin assay so that sensitivity is between 1 and 2 ng/ml.

In the initial footshock and social stress studies conducted during the year, PRL levels tended to be lower than expected from our previous research (Bunnell and Iturrian, 1988), apparently because the time from the initial induction of stress to obtaining the blood samples was on the order of 60-90 minutes and because of habituation to repeated exposures. With chronic or repeated stress, baseline levels of PRL were often near or below the lower limits provided by the RIA kits. By adding standards at the lower end of the curve and by using serum instead of plasma, we increased the sensitivity (to 1 - 2 ng/ml) and reduced interassay variation to about 7%. Reassays of samples which tested below the lower limits of sensitivity of the unmodified commercial kits gave us reliable readings at the lower end of the scale and indicated that PRL was indeed falling to very low levels in the repeated/chronic stressor paradigms.

The RIA kit manufacturer discontinued the kit we were using during the summer of 1988 and substituted a "no spin" kit using cortisol coated tubes. Use of these kits yields slighly, but consistently higher cortisol values from the same samples. Some of the apparent increases in baseline cortisols seen in the data from the later experiments reflect the change in the assay kit used. Otherwise, cortisol values were consistent with what we had seen in our earlier work.

The ACTH assays utilized kits supplied by Incstar Corporation for human ACTH. Recovery ranged from 82-101%; within assay variation averaged 9% and between assay variation

was 6.5%. Sensitivity was about 10 pg/ml. We found that the assay was sensitive to age of the samples as well as the frequency with which they had been thawed and refrozen. The ACTH assay was finally brought on line during the late summer after some initial problems with the procedures were resolved. (We greatfully acknowledge the assistance of E. H. Mougey at WRAIR in this.) The samples which had been obtained earlier in the year and which had already been assayed for cortisol and PRL yielded somewhat lower values than those obtained later and assayed first for ACTH. Baseline ACTH levels in unstressed monkeys ranged between 30 and 80 pg/ml.

c. Blood collection:

In obtaining the blood samples, the monkeys were preadapted to a restraint device used to control them while blood was drawn. Blood samples were collected from the saphenous veins of the animal's legs. During the studies requiring repeated sampling, hematocrits were monitored closely and we found that daily samples of 2.0 ml of blood (more than sufficient to allow us to run duplicate tubes on all three hormones and provide enough cells for the mitogen assays) could be taken from our adult males for several weeks without markedly affecting hematocrit values. In most experiments, daily sampling was not required and we encountered no problems with draws at 2, 3, and 4 day intervals from the females and juveniles used in the studies.

B. Footshock Stress

1. <u>C-Troop Males</u>:

The six males from C-Troop were used in the first footshock experiment. This study was designed to establish the validity and reliability of the immune system assays and characterize lymphocyte responsiveness to mitogens in our species of monkey by using a physical stressor. It was expected that this information would help us evaluate the effects of social stress in the social behavior experiments which followed

The monkeys were placed in individual, ventilated, sound attenuating chambers; white noise was used to mask sounds from the test room. Scrambled footshock was delivered on a variable time ninety second (VT 90-sec) schedule through a grid floor. The sessions lasted 90 min, during which each monkey received approximately 60 shocks. Each 3 sec shock was accompanied by offset of the background white noise and onset of a red cue light. A session began with the shock intensity set at 1.2 ma; this was increasesd to 1.5 ma after 30 min and to 1.8 ma after 60 min to reduce behavioral adaptation to the shock stimuli. Two monkeys were given 5 consecutive days of shock, two received 10 consecutive days, and two got 15 consecutive days.

Animals were loaded into the boxes at 15 min intervals; the staggered starts enabled us to remove each monkey from its chamber and obtain its blood sample immediately after the end of its shock sessions. Blood samples for plasma hormone assays were obtained from the animals on the day before the shock sesions started, both before and after shock on the first day of shock (day 1), and after the shock sessions on days 2, 4, 7, 10, 11, and 15. Samples were taken at weekly intervals thereafter. The baseline samples for the mitogen (Con A and PHA) assays were obtained a month before the experiment began. The mitogen assays were done by the Clinical Immunology Laboratory of the College of Veterinary Medicine using ^{3}H thymidine and equal volumes of fresh whole blood (5 ul).

A summary of the mitogen data obtained from the preliminary footshock study using the C-Troop males is presented in Table 3. In the Table, "Day" refers to the number of days since footshock was begun. On day 4 all 6 monkeys had received four days of shock. On the eighteenth day of the study, 13, 8, or 3 days had elapsed since the animals had been shocked, Thus, the number of days of shock received and time since last day of shock were confounded. The stimulation indexes (SI's) are means of the monkey's responses to the mitogen dose that produced maximum stimulation in each animal on that day.

Table 3

Mean (+/- SEM) Stimulation Indexes* for Con A and PHA Following Footshock. Data From 6 C-Troop Males, April - May, 1988.

	PreShock	Day 4 (Shock)	Day 18	Day 28	Day 35	Day 39
ConA:	326.0	208.3	190.0	122.0	135.3	124.2
	(90.9)	(44.2)	(36.8)	(27.0)	(29.8)	(11.6)
PHA:	401.2	275.7	127.7	248.2	195.5	194.8
	(117.2)	(58.1)	(20.0)	(21.7)	(24.2)	(50.1)

* Stimulation Index (SI):

$$SI = \frac{\text{Counts per Minute + Unstimulated Counts}}{\text{Unstimulated Counts}}$$

One-way ANOVAS (repeated measures) yielded significant days effects for Con A ($F_{5,25} = 4.06$, $p < .01$) and PHA ($F_{5,25} = 2.80$, $p < .05$). Post hoc (Tukey) comparisons showed the SI's for Con A on days 28, 35, and 39 to be significantly lower than the preshock SI. For PHA, the only significant difference was between the preshock day and day 18. Although these tests indicate that something was happening, the

comparisons across days are spurious because of the confounding mentioned in the last paragraph. To provide a more accurate picture of the changes in SI's, the data were examined in terms of number of days since shock had ended. Postshock SI's were converted to percent of control values and aligned by in terms of days postshock. Means are given in Table 4.

Table 4
Mean % of Control SI's Across Days Since Last Footshock Trial

	Preshock Control n=6	Day 4 Shock n=6	PostShock Days: 3 n=2	8 n=2	13 n=4	18-20 n=4	23-25 n=6	29-30 n=4	34 n=2
Con A	100	80.5	88.0	54.5	57.0	41.8	43.8	60.0	73.0
PHA:	100	84.8	39.0	36.0	67.8	83.8	80.0	93.3	51.0

When we examined the data in Table 4 for days on which we had either 4 or 6 data points, there was no obvious relationship between percent change in SI's for either mitogen and the number of days of shock the animals had received.

Footshock produced a decrease in PBL stimulation by the mitogens Con A and PHA; the effects appeared later and lasted longer with Con A than PHA, indicating that the two mitogens stimulated different populations of cells. Beyond this simple statement, interpretation of the data is complicated by the dependence of the SI measure on the unstimulated counts per minute (CPM). A low unstimulated CPM gave a high SI and vice versa. The assays utilized an equal volume (5 ul) of whole blood, and raised the problem that the number of cells present prior to stimulation was not equal for each animal.

Since the C-Troop males were kept in social isolation during the course of the footshock study, no social data were available during this period; however, we did have data on the social rank of each animal prior to the time the shock sessions were begun. With PHA there was a trend toward high social rank being associated with low unstimulated PBL values; the correlations of rho = -.60 for control values, -.79 on Day 4, and -.71 on Day 18 were not statistically significant, however. On the other hand, the positive relationship between social rank and maximum PHA SI, was significant, rho = +.94, on day 4 when all animals had received four days of shock. The control day was +.77 and day 18 was +.62. (Perhaps the loss of the relationship on day 18 reflected the individual differences in the time since the animals were last shocked.)

With Con A, there was a significant negative correlation between social rank and unstimulated PBL values (rho = -.83, p

<.05) and a significant positive correlation between rank and maximum SI (rho = +.89) on day 18. (The unstimulated CPM and SI rho's were -.60 and +.77 on the control day and -.20 and +.69 on day 4.)

Thus, our initial data suggested that the SIs of monkeys of high social rank were less affected by the physical stressor, footshock. The relationships disappeared as the study progressed, however. As noted earlier, animals had received different numbers of days of shock trials and had gone different numbers of days without shock beginning with Day 18. Also, it should be remembered that social testing in these animals had been stopped prior to the beginning of exposure to shock and they had no social contact with each other during this time.

The results of the assays for cortisol and ACTH from samples obtained on Day 1, preshock and postshock, Day 2, postshock, and Day 4, postshock, are given in Table 5.

Table 5
Mean (+/- SEM) Cortisol and ACTH values for C-Troop males (n=6) before (Day 1) and After (Days 1, 2 and 4) Receiving 90 Min Footshock on VT 90 sec Schedule.

	Day 1 Preshock	Day 1 Shock	Day 2 Shock	Day 4 Shock
Cortisol	25.9	33.9	30.0	28.5
ug/100 ml	(1.4)	(2.0)	(1.6)	(1.6)
ACTH	72.8	83.1	76.7	69.1
pg/ml	(5.4)	(4.4)	(9.3)	(7.2)

A one-way ANOVA of the cortisol data was significant ($F_{3,15}$ = 7.70, $p < .01$) and the aposteriori tests indicated that the Day 1 shock values were significantly elevated over those for Day 1 preshock and Day 4 shock. This indicates that there was habituation of the cortisol response across 4 days of 90 min shock sessions. The pattern for ACTH was similar, but variability was relatively large and the ANOVA gave a nonsignificant F = 0.95. The age and condition of the samples at the time they were assayed (see the earlier discussion of the hormone assays) may have contributed to these results. It is also likely that ACTH levels were dropping from peak values following 90 min of exposure to the stressor.

The problems with the prolactin (PRL) assav described in Section A4, above, were discovered durinq the shock phase of the experiment. Values were very low prior to shock on Day 1 and went below the sensitivity of the assay for many animals durinq this phase of the study. (Reassay of the plasma samples with an additional standard added at the lower end of the curve indicated that the low values in response to the physical stressor were probably real and that 90 min of footshock was depleting PRL. At this point, we shifted from plasma to serum, and obtained the higher values during the postshock days that are described in the next paragraph.)

When we examined the PRL data obtained from the modified assay procedure using serum samples from days 18, 28, and 36 we found that PRL values were slightly higher in all 6 monkeys on day 28 as compared to day 18. SI's were lower for Con A in 5 of 6 animals and higher for PHA in all 6 animals on day 28 compared to day 18. On day 35, PRL levels were significantly elevated ($F_{2,10} = 9.68$, $p < .01$), perhaps due to the presence of a new caretaker who participated in the collection procedure for the first time and may have been a mild stressor. On day 35 there were no relationships between PRL levels and SI's for either mitogen. PRL levels tended to be slightly lower than baseline throughout the shock experiment, indicating that the system had habituated to the shock situation rather quickly. Table 6 compares the mean PRL values on days 18, 28, and 35 with baseline values obtained from 5 samples from these animals in June and July - serum was assayed in each case. During this period, the animals were undergoing retraining on an operant schedule and were being observed in social interactions several times a week. The samples were obtained prior to that day's training or social observations. The rather large standard errors are due to one animal whose PRL levels, except on 7/8, were consistently 2-3 times higher than those of the other monkeys.

Table 6

Mean (+/- SEM) Prolactin Values (ng/ml) in C-Troop May-July, 1988.
(n = 6 monkeys per day)

Date:

5/6 Day 18	5/16 Day 28	5/23 Day 36	6/13	6/23	6/29	7/8	7/14
7.7 (3.9)	10.6 (4.0)	16.0 (6.3)	13.7 (5.3)	12.7 (3.5)	14.0 (7.5)	8.7 (1.5)	16.6 (7.0)

2. I-Troop Males:

The first footshock study served to familiarize us with the mitogen assays, helped us characterize lymphocycte responsiveness to mitogens in our monkey species, and allowed us to examine the way this system responded to a physical stressor. However, there were a number of issues that could not be resolved by the preliminary study and we needed to do another footshock experiment to enable us to address some of these questions directly. The second study used the same number of shock days for all animals, increased the number of blood samples obtained for both the mitogen and the hormone assays throughout the experiment, and was conducted with members of a social group (I-Troop) such that social behavior was monitored continuously during the shock and postshock days. All animals received 13 consecutive days of footshock, using the same procedures for administering shock as in the preliminary experiment. Blood samples for immune function and hormone assays were obtained at four day intervals, beginning eight days before the shock sessions were started and continuing through Day 26 postshock. Additional samples were obtained for the hormone assays every two days during the time the animals were receiving shock in order to monitor PRL habituation to the physical stressor. Following each day's shock session, the animals were kept inside for 90 min before being placed back in the social group. Social observations were done after the group was reunited each day. No experimental manipulations of the social situation were conducted during the experiment since one of the objectives was to look at individual differences in response to the physical stressor as a function of the animals' basal social behavior and social status. However, some spontaneous changes in rank did occur and these will be discussed below.

On the following pages, Figures 2 - 6 give the results of the mitogen assays across 11 data points. (Postexperiment samples were obtained at three time points in November to verify the return to baseline.) In Figure 2, the initial number of circulating lymphocytes in 1 ul of fresh blood prior to mitogen stimulation is given for each of the six monkeys. It will be seen that there is considerable individual variability in the data, although each animal exhibited a depression at some point during the shock trials, followed by an adaptation. At the end of shock, the count overshot the baseline values and then recovered to slightly below baseline. This pattern can be seen in Figure 3, which gives the mean values for the group.

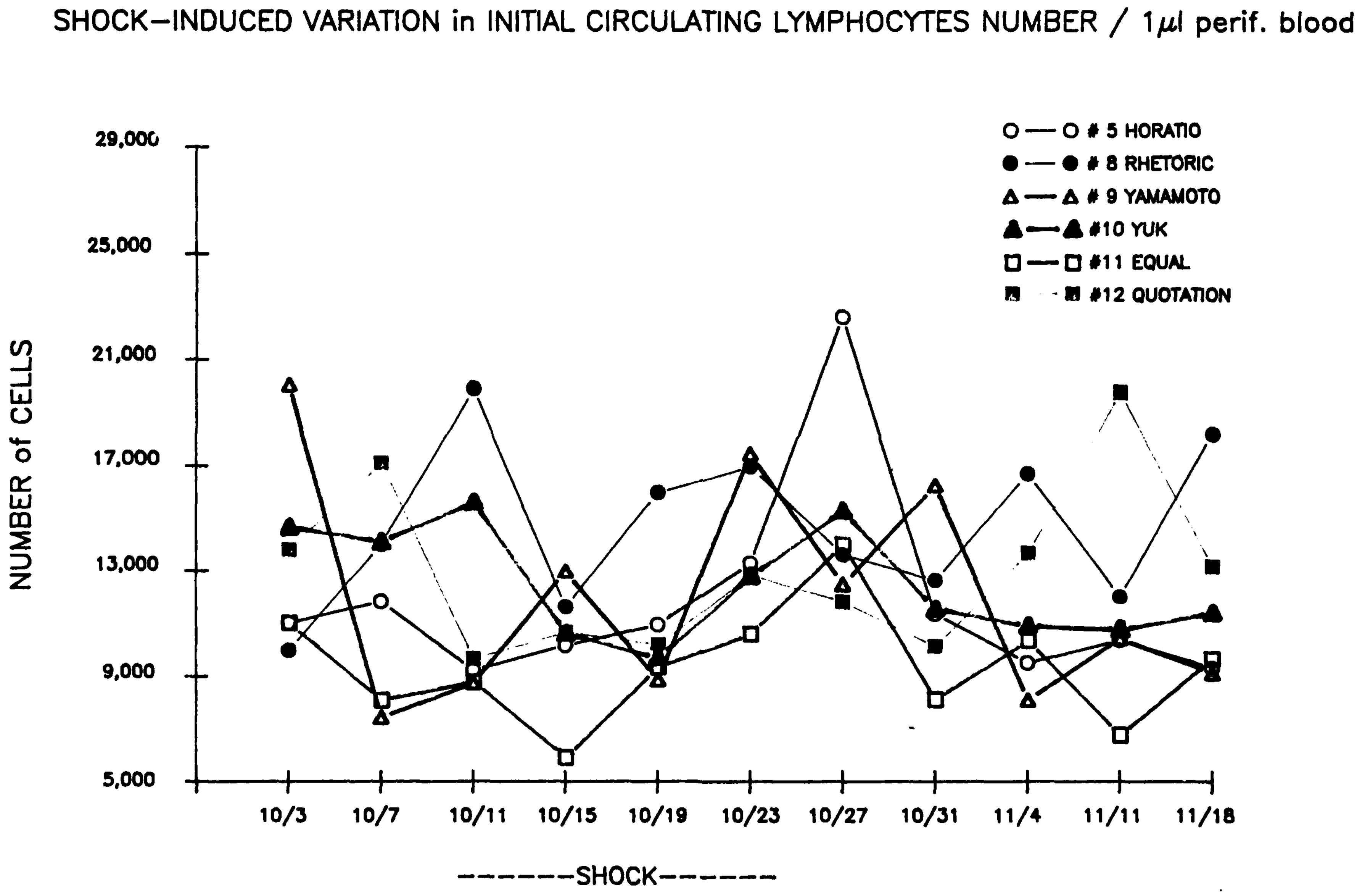


FIG. 2: INITIAL CIRCULATING LYMPHYOCYTE NUMBER AND THE EFFECTS OF SHOCK FOR INDIVIDUAL MONKEYS OF I TROOP

SHOCK–INDUCED VARIATION of INITIAL CIRCULATING LYMPHOCITE NUMBER / 1 μl PERIF. BLOOD

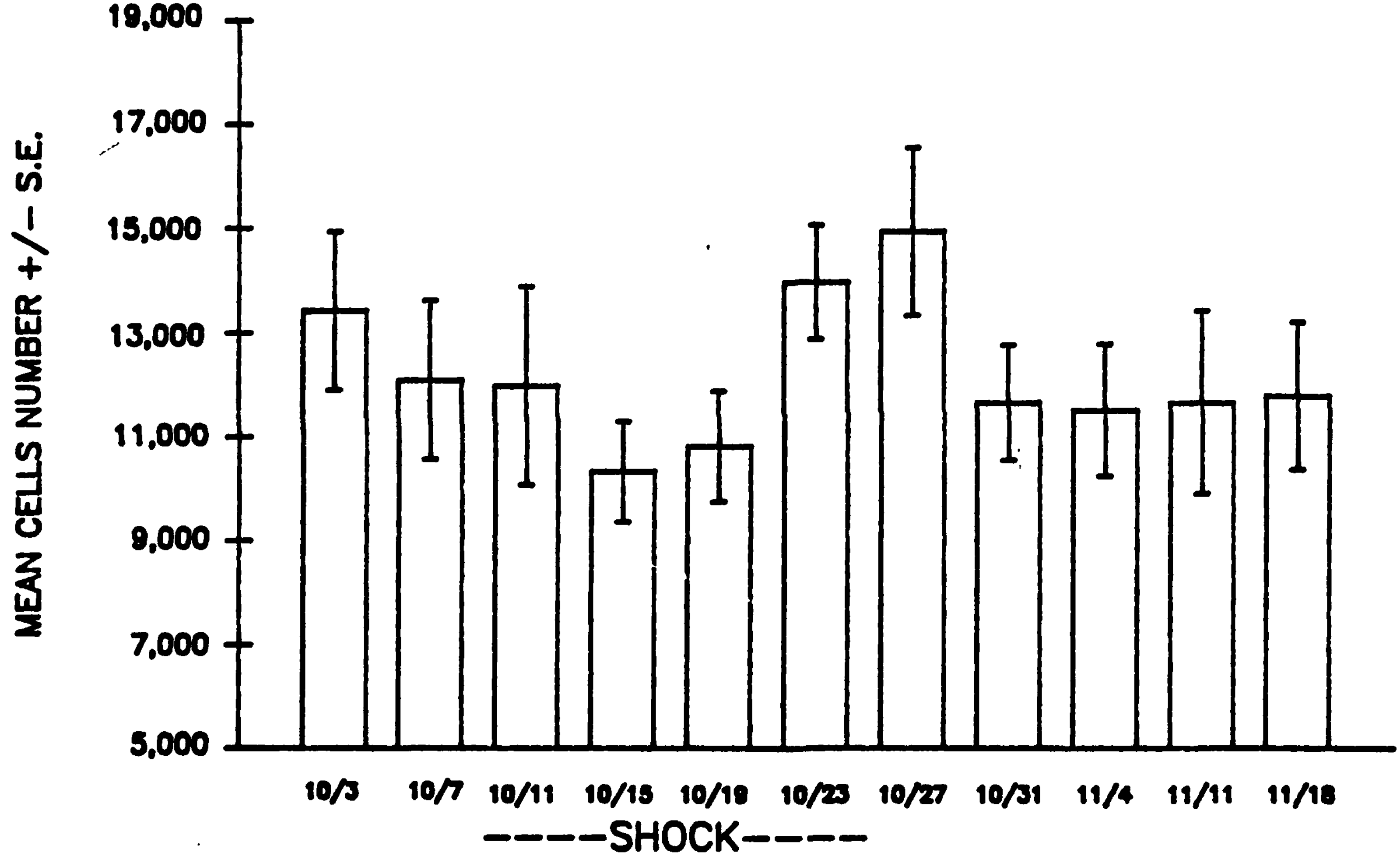


FIG. 3: MEAN INITIAL CIRCULATING LYMPHOCYTE NUMBER FOR I TROOP

Shock–induced alteration of Lymphocyte reactivity to Con A

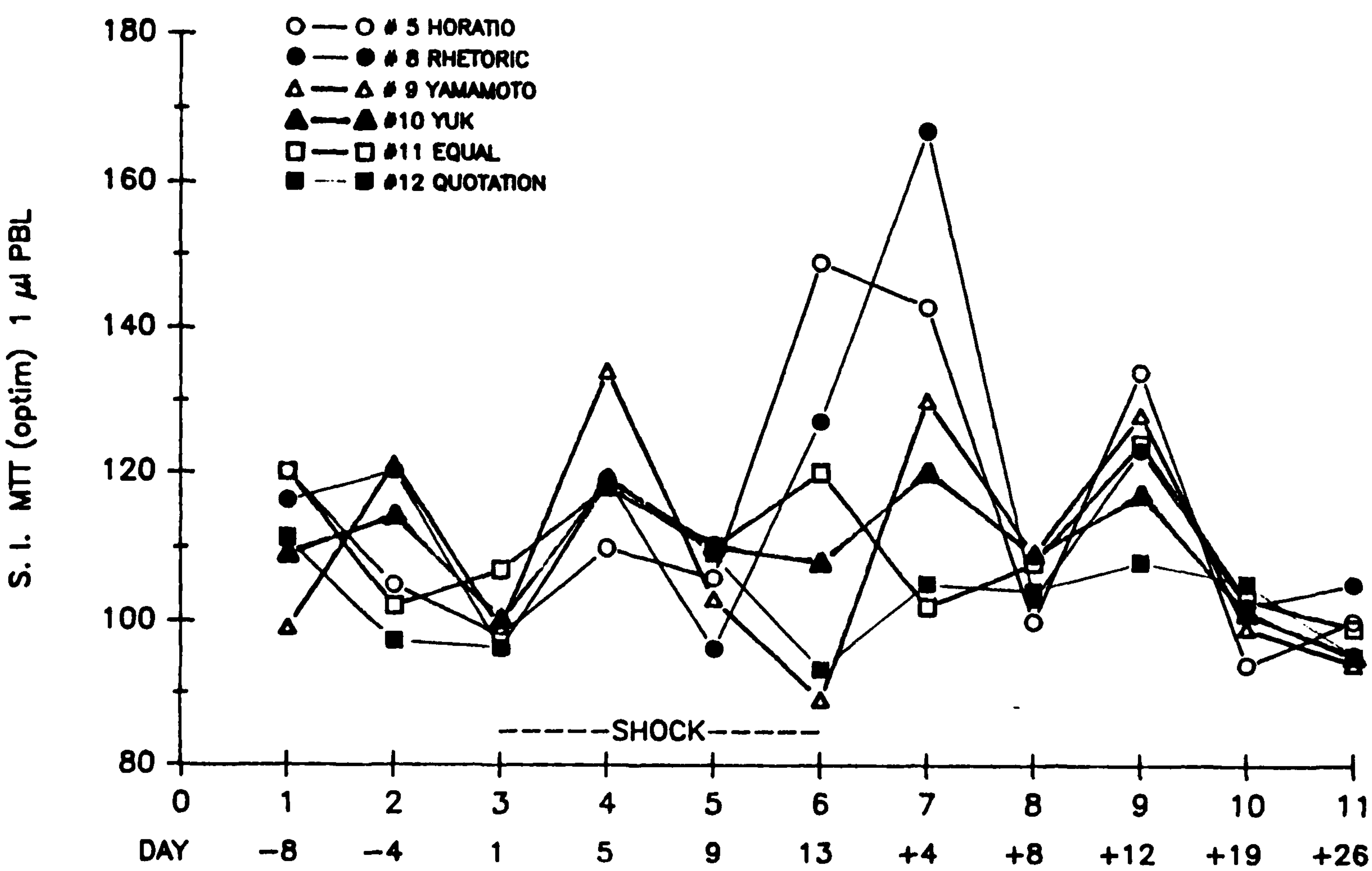


FIG. 4: SHOCK-INDUCED ALTERATION OF OPTIMAL LYMPHOCYTE REACTIVITY TO CON A. FOR INDIVIDUAL MONKEYS OF I TROOP

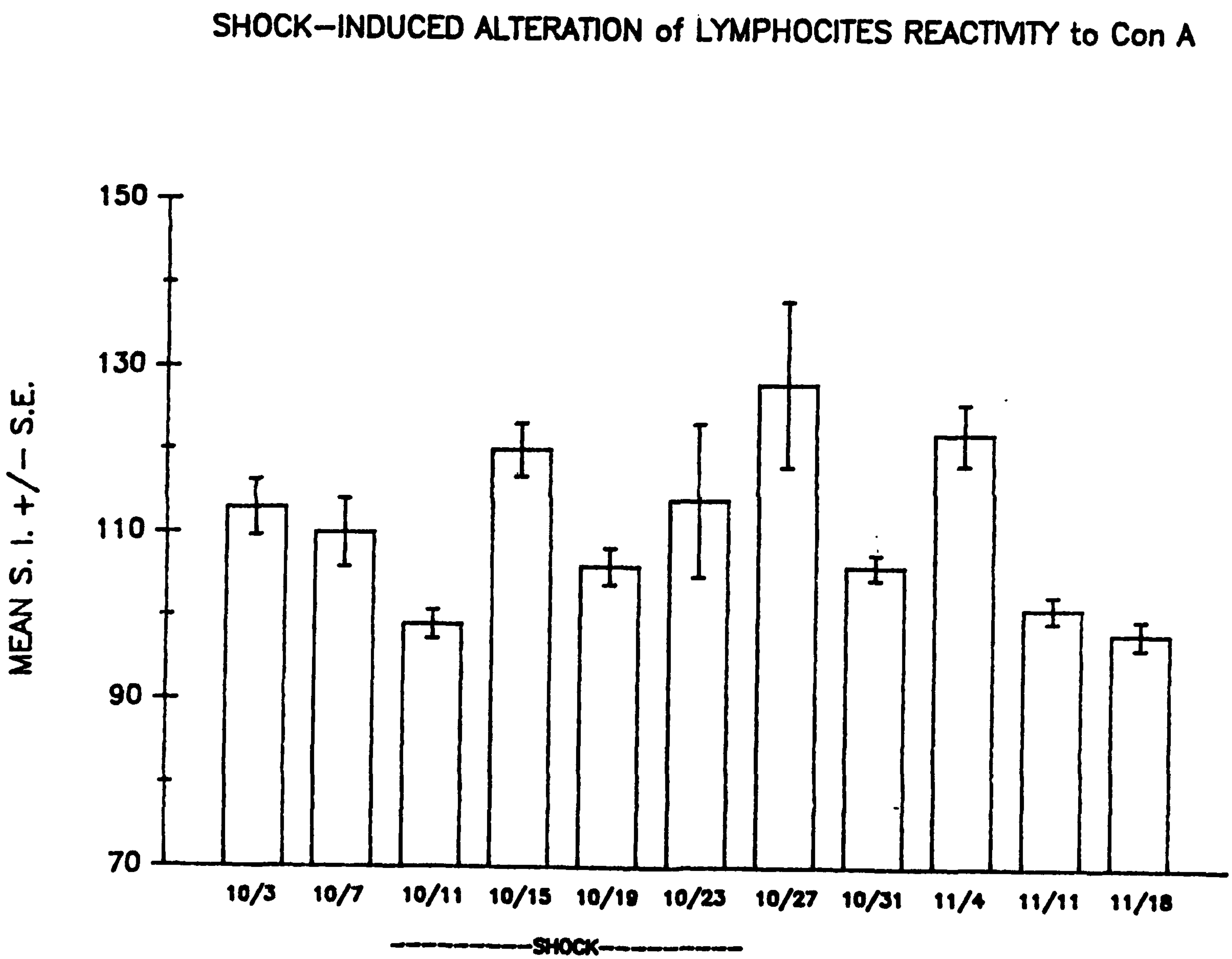


FIG. 5: MEANS OF I TROOP SHOCK-INDUCED ALTERATION OF LYMPYOCYTE REACTIVITY TO CON A

Shock–induced alteration of Lymphocyte reactivity to PHA

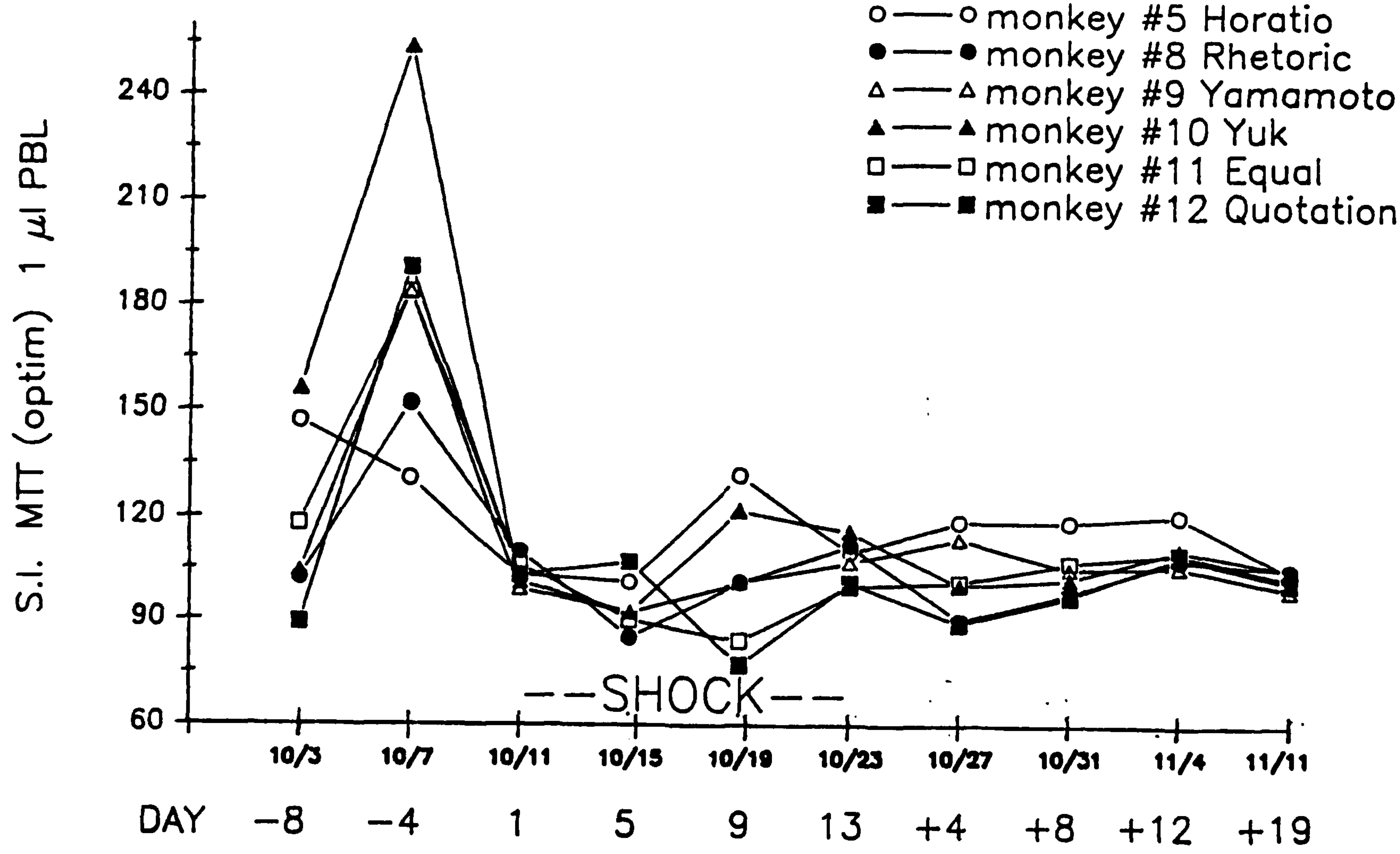


FIG. 6: SHOCK INDUCED ALTERATION OF PBL REACTIVITY TO PHA FOR INDIVIDUAL MONKEYS OF I TROOP

Because of the individual variations in the patterns, the statistical analysis across days was not significant. However, comparing the fourth shock day (10/15) and the first postshock data point (10/27) with a pooled baseline mean obtained from combining the two preshock days and the last four postshock days showed PBLs to be sigificantly depressed on 10/15 ($F_{1,14}$ = 4.61, p < .05) and elevated on 10/31 ($F_{3,20}$ = 3.10, p <.05). This finding, while not robust, suggests that initial PBL count may be a useful indicant in future studies.

The individual patterns of reactivity to Con A stimulation are shown in Figure 4, as are the means in Figure 5. The data are presented as SIs obtained from the MTT assay procedure and represent the optimum stimulation concentration of the mitogen. SI's were low on the very first day of shock and were highest four days after shock was terminated. Because of the large variances, the overall statistical analysis was not significant. Variability was greatest on the last shock day and four days post shock, suggesting that repeated shock experiences magnified differences in individual patterns of response. The presence of a normal 8-day rhythm, which was partially disrupted by the shock experience, is detectable in both the individual and group data. Some sort of individual time series analyses of baseline data across many weeks would be necessary in order to confirm the presence of such a pattern. If such a cycle exists, it could be an important determinant of the magnitude, and even the direction, of the immune system responses produced by experimental manipulations of the animals' environment.

One finding of potential significance emerged when the data in Figure 4 were examined in terms of the events occurring in the social situation. Two monkeys reversed their ranks twice during the 13 days. Horatio was ranked second (below Yuk) and Yamamoto was third for several weeks prior to the study. During the first four shock days, they switched ranks and Horatio submitted to Yamamoto; during the last four shock days they reversed again. (The encounters in which the reversals took place were not observed, so the amount of aggression that occurred is unknown.) Immediately following the first reversal, Yamamoto had the largest increase in his SI to ConA and Horatio the smallest (Shock Day 5 data point); after the second reversal occurred, Horatio had the highest SI and Yamamoto the lowest (Shock Day 13 data point). It appears that winning out is associated with higher SIs and losing with smaller increases, or even a decrease. Rhetoric, who had an increased SI on Shock Day 13 had a very high score on Postshock Day +4. As well as being the youngest monkey in the group, Rhetoric was a social outcast who submitted to all the other members of the troop and engaged in very few affiliative behaviors; he was also an epileptic and suffered a tonic-clonic seizure on the fourth day of shock. However, except for the abnormally high SI

on Postshock Day +4, his scores were within the normal range. Aside from Rhetoric, Quotation was the lowest ranked monkeey and he had the lowest mean SI of the group across all days (X = 103.7, SEM = 3.3). Yuk, the alpha male, had an intermediate mean SI (X = 109.5), but his scores across all data points were the least variable (SEM = 2.4). The results were quite similar to those for C-Troop in the first study; however, the correlation between rank and variance was not sigificant because of the fluctuations in the SIs of Horatio and Yamamoto described above.

Figure 6 presents the individual data for PHA stimulation. The sharp increase in SIs observed in 5 of the 6 animals on the baseline day four days prior to beginning the shock sessions (10/7) is an anomoly for which we have no ready explanation. Social behavior was quite stable on this day and there were no other events which might account for the change. The MTT assay data showed none of the pattern obtained with the thymidine assay in the C-Troop study. Although there was a near perfect correlation between rank and SI in response to PHA eight days before shock (Preshock Day -8), the relationship disappeared on Preshock Day -4. About the only thing that can be said is that PHA is stimulating a different population of cells than ConA.

The individual and group social behavior frequencies in the aggressive, submissive, and affiliative categories are presented in Table 7.

Table 7

Social Behavior in Six I-Troop Males Before, During, and After Footshock. Data are Mean Frequencies per Day

<u>Aggressive</u>

Animal	Rank	Pre-Shock (Five Days)	Shock (First Five Days)	Shock (Last Eight Days)	Post-Shock (Four Days)
Yuk	1---	0.3	0.8	0.5	0.3
Horatio	232-	0.0	0.4	0.0	0.3
Yamamoto	323-	0.4	1.0	0.1	0.5
Equal	4---	0.0	1.0	0.3	0.0
Quotation	5---	0.0	0.4	0.1	0.0
Rhetoric	6---	0.0	0.0	0.0	0.0
	Mean (+/-SEM)	0.1 (0.08)	0.6 (0.16)	0.2 (0.08	0.2 (0.09)

Table 7 (Continued)

Submissive

Yuk	0.0	0.0	0.0	0.0
Horatio	1.0	0.2	0.1	0.0
Yamamoto	0.2	0.8	0.1	0.0
Equal	1.8	1.6	1.0	1.0
Quotation	0.8	3.0	1.9	1.2
Rhetoric	6.4	5.4	0.9	3.5
Mean	1.7	1.8	0.7	1.0
(+/-SEM)	(0.98)	(0.84)	(0.30)	(0.56)

Table 7 (Continued)

Affiliative

Yuk	2.8	6.0	0.9	2.5
Horatio	3.2	6.4	0.9	5.0
Yamamoto	11.0	16.6	4.6	7.0
Equal	14.6	24.2	5.1	12.3
Quotation	8.0	11.8	4.3	7.3
Rhetoric	0.6	1.2	2.1	0.3
Mean	6.7	11.0	3.0	5.7
(+/-SEM)	(2.2)	(3.4)	(0.8)	(1.7)

These data provide the second finding of interest in the social behavior data from the I-Troop shock study. In the group, there was an increase in both aggressive and affiliative behaviors over preshock levels during the first five days of shock; the affiliative behavior scores dropped to very low levels during the last 8 days of shock and then began to recover during the first postshock week. The overall one-way ANOVAs of aggressive and affiliative scores were statistically significant ($F_{3,18} = 7.70$, $p < .01$ for aggressive and $F_{3,18} = 7.78$, $p < .01$ for affiliative). There was a similar trend toward a reduction in submissive behavior over the last 8 shock sessions, but it was not significant. Post hoc tests (Tukey) between means indicated that the mean aggressive score on the first 5 days of the shock period was significantly elevated over the other three means. The mean for the first five days of shock for affiliative behavior was significantly higher than that for the last 8 days of shock and for the postshock period. (Note: had Rhetoric not been included in the analyses, the overall Fs for aggressive and affiliative (but not submissive) behaviors would have been larger and the preshock mean of the affiliative scores would have been significantly lower than the mean for the first five days of shock.)

In terms of lymphocyte response to ConA mitogen stimulation, the increasing aggressive and affiliative behavior frequencies are associated with rising SIs (Shock Day 5, Figures 4 and 5), depressed social scores with falling SIs (Shock Day 9 and, to a lesser extent, Day 13), and increasing affiliative behavior scores with a rise in SI on postshock day +4. The changes in social behavior accompanying footshock are fairly clear and the relationship between affiliative scores and the direction of changes in SIs is very interesting and needs to be examined in further experiments.

Hormone data for the two preshock data points and for samples drawn every other day immediately following the shock sessions are given in Table 8.

Table 8

Cortisol and Prolactin Levels Prior To and During the First Nine Days of Shock in the I-Troop Shock Experiment.

Cortisol (ug/100ml)

	Preshock		Shock				
Day:	10/3	10/7	10/11	10/13	10/15	10/17	10/19
Mean	55.9	56.6	53.2	45.4	45.2	38.5	41.9
(+/- SEM)	(3.7)	(2.3)	(4.7)	(4.2)	(2.4)	(3.3)	(2.7)

Prolactin (ng/ml)

Mean	17.8	13.1	3.1	3.6	5.2	3.7	3.5
(+/- SEM)	(7.2)	(4.1)	(0.2)	(0.4)	(1.9)	(0.6)	(0.5)

The hormone data are difficult to interpret, primarily because of the high values on the two preshock days. In the case of cortisol, all of the values are elevated relative to what we had been used to seeing. Some of this may be due to the use of the new assay kit described earlier, but we still would have expected an increase over the first few shock days when, in fact, we found a significant decrease ($F_{4,30} = 5.66$, $p < .001$) when the animals were shocked. Days 10/17 and 10/19 were significantly lower than days 10/3 and 10/7 on the posthoc Tukey tests; the first day of shock (10/11) was also higher than 10/17 according to the a posteriori test. Although it seems unlikely, the high preshock scores suggest that the animals were imperfectly adapted to the handling procedure at the beginning of the experiment or were responding to some unknown change in the procedure.

Although the same problem may be present in the PRL data, the data are much more useful. The high baseline values are not out of line with baseline levels obtained with C-Troop (see Table 6) although the standard errors are large on the preshock days. Because of the large variance, a square root transformation was used on the prolactin data. The ANOVA indicated that the depression in PRL levels during the shock sessions was significant. ($F_{4,30} = 7.50$, $p < .001$). It seems clear that 90 min of footshock on the first and subsequent days depresses serum PRL and confirms the trend found in the C-Troop shock study. Examination of rat data obtained by Kant, et al (1982) indicates that high levels of PRL obtained after 15 min of footshock (as well as other stressors) were attenuated with 30 and 60 min of the stressor. An important next step will be to sample at intervals throughout the shock sessions to determine the intrasession time course of the PRL response.

C. Social Behavior and Social Stress

The primary work on social behavior was done with the I-Troop males and with NT-Troop, one of the large breeding troops. The work with each troop will be described separately, after which some of the data from the two studies will be presented in combined form to illustrate the effects of social manipulations on the measures of immune function.

1. I-Troop Males:

The I-Troop males were removed from their compound in January, 1988 and placed in individual cages in the laboratory building in January. Two animals died between January and June, when the troop was reformed from the four surviving monkeys. Two young males (Rhetoric and Lucifer) were also removed from NT-Troop during the winter and kept in the laboratory during this period. They, together with one of the C-Troop males (Horatio), were introduced into I-Troop following reformation of the troop.

On each day following reformation of the group, social behavior was scored first, after which the monkeys were brought into the laboratory to be weighed, a blood sample taken if scheduled, and the four original members of the troop and one of the NT-males (Lucifer) were placed in test chambers for performance testing on a MULT RI 1-min RI 1-min reinforcement schedule. Social data were obtained using forty min general scans except on days when new animals were introduced to the group. On these days, 20 min of scan data were obtained before the new animal was placed in the group; after the introduction there was a 40 min scan after which all animals were removed from the group and blood samples obtained. Because the sampling procedure required that two samples be obtained, one for

mitogens and one for hormones, sampling took an average of about 10 min per animal. Since the blood draws were usually started about 15 min after social testing this meant there was a delay of as much as 75 min between the time an animal was removed from the social group and the time its sample was obtained. The order in which the monkeys were sampled was changed each time to minimize any bias introduced by the delays; nevertheless, the presence of differences in the delay intervals could have operated to obscure small, but perhaps real differences, particularly in hormone responses. The SI data from this experiment were obtained from ^{3}H thymidine assays performed by the Clinical Immunology Laboratory on a fixed volume (5 ul) of heparinized whole fresh blood.

A baseline blood sample for assays of response to the mitogens ConA and PHA was obtained from each monkey four days before the group was reformed. Except for a gap during the second week of July, samples were taken twice a week throughout the experiment. There was little agonistic behavior in the group during and immediately following the reformation and, because we were anxious to obtain pilot data on the possible effects of social group manipulations on mitogen indicants of immune function, we went ahead with the introduction of the first "stranger" (Rhetoric) to the troop three days later. The five member troop was monitored for three weeks during which there was considerable social instability and an increase in aggressive behavior. Although he was not severely injured during the introduction, Lucifer, the second stranger introduced into the group went into shock on the afternoon following his introduction and could not be revived. One week later, Horatio, an older and larger monkey from C-Troop, was introduced and the social behavior and mitogen responses of the six member troop were monitored for another 18 days.

The stimulation indexes for ConA and PHA during the course of the study are summarized in Table 9. Figure 7 contains the same summarized ConA data expressed as the optimal increase in CPM for ^{3}H thymidine uptake.

Table 9

Stimulation Indexes for Con A and PHA During Social Group Manipulations of I-Troop, June-August, 1988. Data for Animals not yet in Group are in Parentheses.

Con A

Date:	6/20	6/24 Group Reformed	6/27 Rhetoric Intro	7/1 Yuk Hurt	7/5 Yuk Hurt	7/15	7/18 Lucifer Intro; Yuk Out
Animal:							
Equal	(160)	133	58	62	43	267	72
Yuk	(178)	111	68	201	181	91	(211)
Yamamoto	(189)	98	69	96	46	90	142
Quotation	(148)	224	89	52	no sample	188	162
Rhetoric	(74)	(71)	201	201	63	122	159
Lucifer	(93)	(42)	(38)	(80)	(29)	(125)	298

Date:	7/22 Lucifer Dead	7/25 Horatio Intro	7/29	8/1	8/5	8/8	8/12
Animal:							
Equal	95	72	83	52	30	67	250
Yuk	148	192	169	216	96	228	143
Yamamoto	132	62	108	100	49	33	48
Quotation	271	320	320	421	90	261	108
Rhetoric	132	271	118	192	209	138	161
Horatio	(147)	272	169	148	112	80	75

PHA

Date:	6/20	6/24 Group Reformed	6/27 Rhetoric Intro	7/1 Yuk Hurt	7/5 Yuk Hurt	7/15	7/18 Lucifer Intro; Yuk Out
Animal:							
Equal	(218)	89	66	124	43	259	82
Yuk	(263)	155	64	289	236	68	(181)
Yamamoto	(176)	92	119	151	306	155	96
Quotation	(189)	291	116	98	no sample	209	155
Rhetoric	(65)	(66)	197	152	113	127	184
Lucifer	(78)	(65)	(120)	(77)	(67)	(133)	119

Date:	7/22 Lucifer Dead	7/25 Horatio Intro	7/29	8/1	8/5	8/8	8/12
Animal:							
Equal	230	115	112	329	39	83	181
Yuk	121	123	182	148	113	258	288
Yamamoto	181	282	225	329	56	123	116
Quotation	301	335	318	658	239	350	119
Rhetoric	116	168	203	230	109	198	265
Horatio	(241)	255	77	174	38	252	110

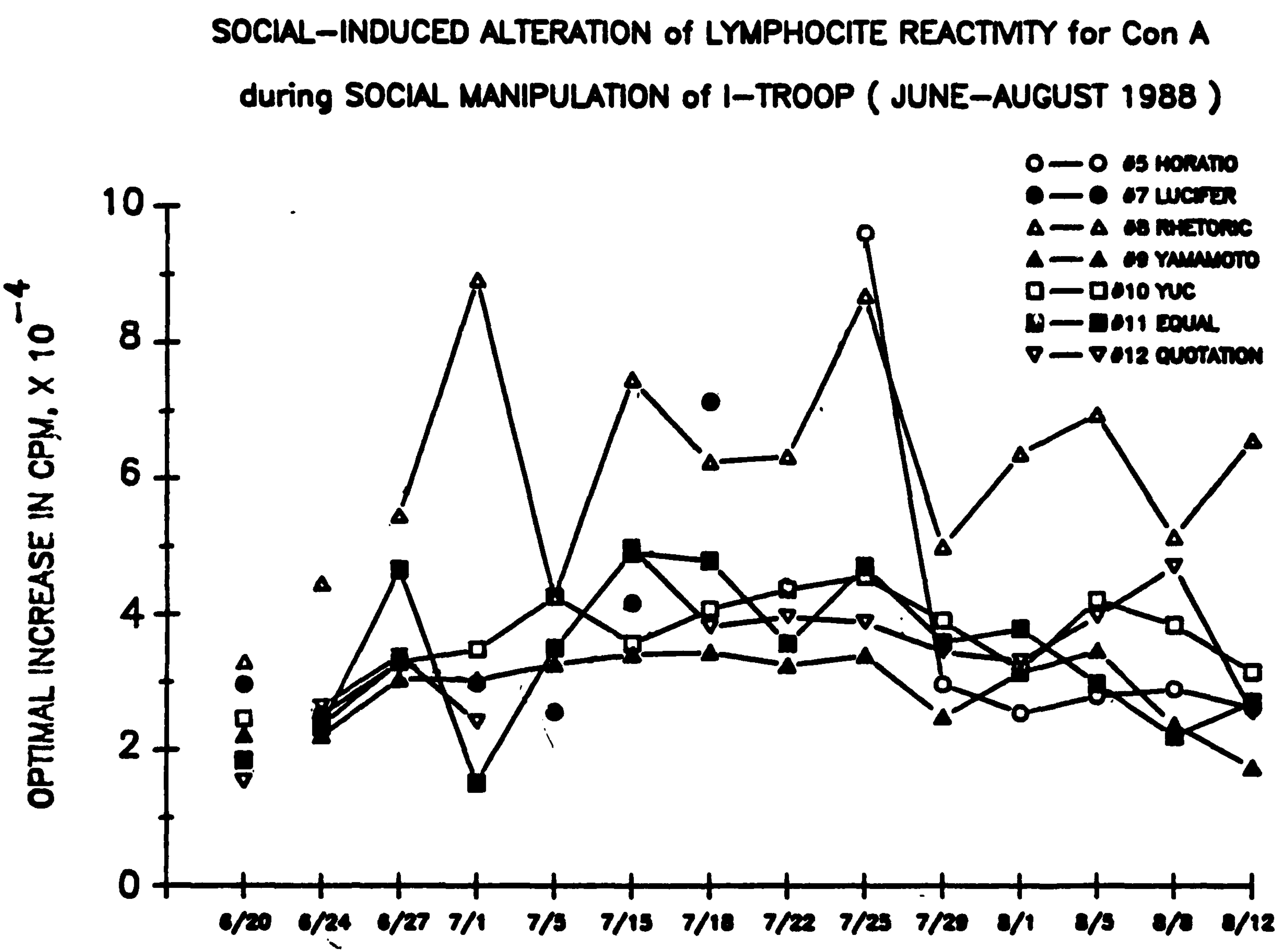


FIG. 7: ALTERATION OF INDIVIDUAL MONKEYS PBL REACTIVITY TO CON A DURING SOCIAL MANIPULATION OF I TROOP

When the first four monkeys were placed together after a separation of almost 5 months, three animals had lowered SI's to both mitogens on the very first day; the fourth (Quotation) showed increases. Quotation ranked fourth in the social hierarchy (he is also an animal that suffered from chronic diarrhea of undeterminable origin). Although Equal had been the top ranking male prior to the separation of the animals five months before, Yuk established himself as the alpha male when the group was reformed. There was no aggression and only seven submissive responses were recorded during the three days prior to the introduction of Rhetoric. At the time Rhetoric was introduced, all four members of the original group, including Quotation, had reduced SI's. Rhetoric had relatively low SI's prior to his introduction. When Rhetoric was put into the group he was subjected to a large amount of aggression by the other monkeys. He responded to the 130 aggressive behaviors directed toward him with 4 aggressive and 70 submissive behaviors in the 40 min session. He submitted to all four original members of the group and avoided all social contact with them during the next four weeks of observations. His SI's went up sharply and with one exception (Con A on 7/5) remained slightly to moderately above preintroduction levels. The social rank structure was unsettled for the three weeks following Rhetoric's introduction. Yuk was slightly injured on July 1 and again on July 3 when he was removed from the troop to have a wound sutured; returned to the troop on July 5, he was injured again on the afternoon of July 15 and was not in the group when Lucifer was introduced. Yuk had elevated SI's on 7/1, 7/5 and 7/18. There was a major shift in the rank structure during this time as Equal replaced Yuk as the alpha male; Yuk managed to hold second rank above Yamamoto.

Lucifer's introduction produced another increase in agonistic behavior in the troop. Equal, Yamamoto and Quotation directed 116 aggressive behaviors toward Lucifer who responded with 12 aggressive and 47 submissive behaviors. In this session, Rhetoric was the recipient of 12 aggressive behaviors from Equal and Yamamoto and responded with no aggressive and 13 submissive behaviors. Although Lucifer had not received any serious wounds and seemed to be adapting to the situation, he went into shock several hours after the introduction and we were unable to revive him. His Con A SI, but not his PHA SI, was elevated on the day of the introduction.

One week after Lucifer's introduction, Horatio, normally a member of C-Troop, was placed in I-Troop. (Yuk had been returned to the group 4 days before.) Once again there was an increase in agonistic behavior, but Horatio held his own much better than had Lucifer or Rhetoric. Horatio made 41 of the 56 submissive responses observed during the introduction and submitted only to Yuk, Yamamoto, and Equal. He directed his 14 aggressive behaviors toward the same three monkeys who

reciprocated with a total of 124 aggressive behaviors. Quotation did not participate in the agonistic encounters at all and Rhetoric made 15 submissive responses to the top three animals. Affiliative behaviors were very low throughout the introduction and Horatio made no affiliative responses although he made one attempt to enlist Yamamoto. Over the next few days Horatio established his dominance over Rhetoric and Quotation and later moved above Equal who fell to fourth rank. Yuk again became the alpha male. In contrast to Rhetoric who showed very little affiliative behavor toward the other members of the group within the first month after his introduction, Horatio began to show affiliative behavior by the second day following his introduction and was well integrated into the social organization within the first week.

Horatio's SI to Con A was elevated over control values on the day he was introduced and gradually decreased over the next five data points. The SI's are quite low by the end of the study, but are similar to the values obtained from him on days 28, 35, and 39 of the C-troop shock study (above). His SI for PHA stimulation did not change on the introduction day. (The SI's we obtained for him during May were very close to his preintroduction SI of 241). For the remainder of the study Horatio's PHA SI's fluctuated between being very low and baseline levels on alternate measures. This pattern in Horatio's immunological response to the social introduction was evident even when the data were expressed as changes in counts per minute across all four doses of both mitogens (Figure 8). Both Rhetoric and Yuk received minor cuts on the day of Horatio's introducton and Quotation was being treated with neomycin for diarrhea during the last week of July. Some of the fluctuations in SI's during late July and early August might be due to the changing social situation as Horatio moved up and Equal moved down in the dominance hierarchy, but the changes in SI's were not highly correlated with the social behavior scores.

The I-Troop data may be summarized as follows: Reformation of I-Troop produced depressions in ConA and PHA mitogen stimulation indexes (SIs) in the four original troop members. This also occurred in the C-Troop male, Horatio, that was introduced later, but not in the NT-Troop stranger, Rhetoric, that had low SI's prior to his introduction. The third stranger, Lucifer, did not survive long enough to provide post introduction SI data but, like Rhetoric, his SI's prior to introduction were lower than those of the original troop members. It should be noted that Alien, another NT-male that was removed from the troop along with Rhetoric and Lucifer, also had low SI's while in the laboratory. We don't know if these low values were typical of these young males (their mean age was 6.7 years compared with 17.2 years for the older monkeys) or a consequence of their removal from their social group. A experiment to examine this has been proposed in the plan for continuation of the project.

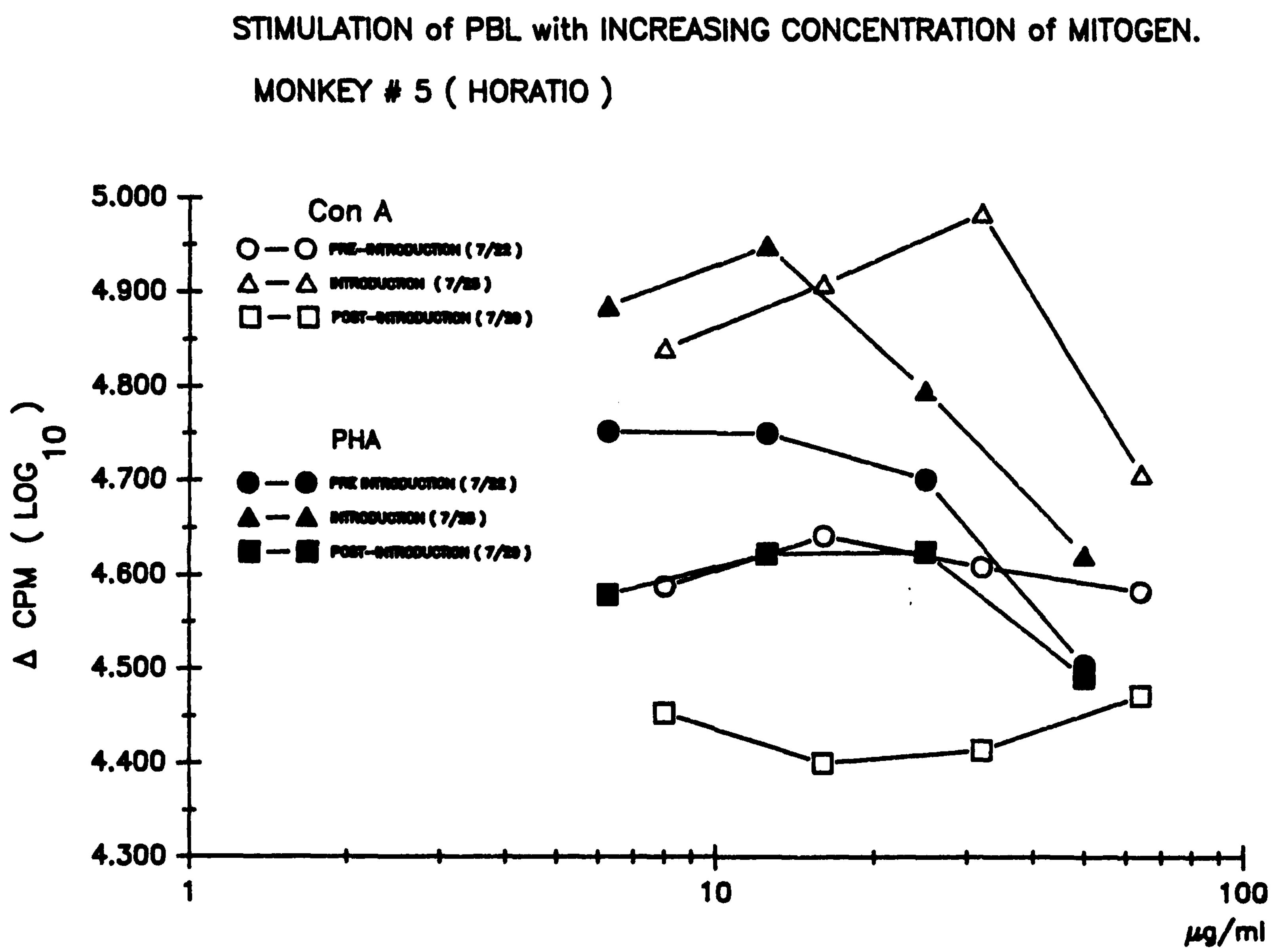


FIG. 8: ALTERATION OF MITOGEN DOSE RESPONSE OF AN INDIVIDUAL MONKEY DURING SOCIAL MANIPULATION OF I TROOP

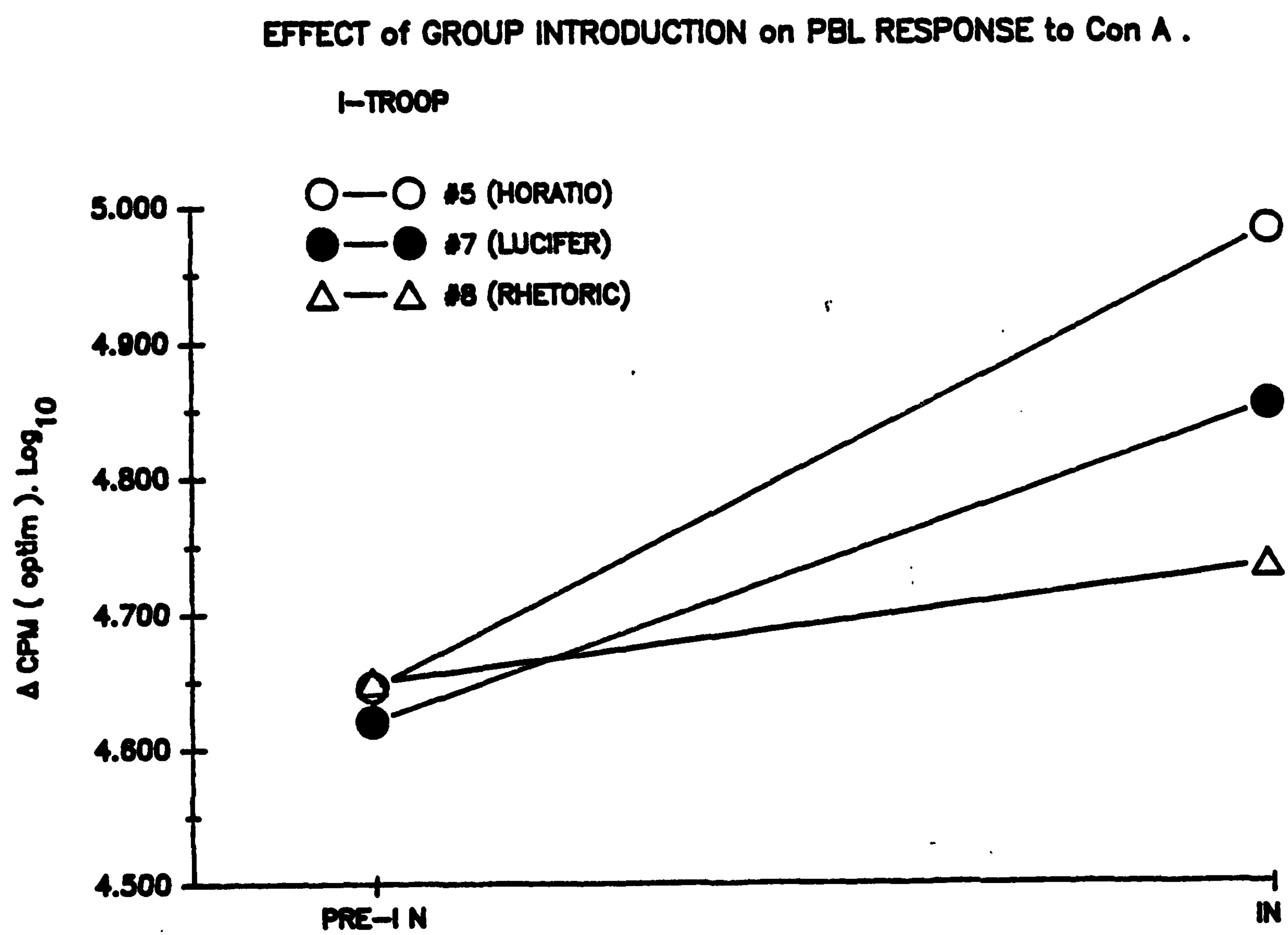


FIG. 9: EFFECT OF INTRODUCTION INTO I TROOP ON PBL RESPONSE TO CON A.

All three strangers exhibited large increases in the ConA SI's on the day each was placed in the group. This is illustrated on the log scale of changes in counts per minute shown in Figure 9. (Quotation, the lowest ranking monkey of the original four also had a moderate increase in his SI to ConA on the day the group was reformed.) The three strangers had received a few cuts during their initial interactions with other members of the troop. Similarly, Yuk showed an increase in SI's on the days he was injured. The data for PHA were less clear; the SI's of Quotattion, Rhetoric and Yuk were up following injury, while Lucifer did not change.

If one follows the data for individual monkeys (Table 9), several show a periodicity of about eight days in their responses to mitogen stimulation. We have not examined this mathematically since there are too many confounding factors, but the data suggest that it would be worth looking for such rhythms in future experiments. If such patterns are real, they might be endogenous, they might involve physiological factors such as age or hormone levels, or they might be related to social variables such as rank, degree of integration into the group, and levels of agonistic behavior. In any event, the effects of an experimental manipulation on immune function might be expected to vary depending on the phase angle in the cycle at the time the manipulation is made.

The four adult males from I-Troop (plus Lucifer and Alien from NT-Troop) were trained on a multiple RI RI 1-min operant schedule. Operant testing was done in the mornings, after social testing and blood sampling and before the morning feeding so that the monkeys were fasted overnight. As an illustration of the effects of social manipulations on performance, the total responses of the four original I-Troop males during the group reformation and the successive introductions of three strangers are shown in Table 10

There were considerable individual differences in overall responding, but response patterns tended to be consistent within animals. There were no changes in performance when the group was reformed (if anything, there was a slight facilitation associated with being back in the group), but the introduction of strangers generally produced a depression of performance that was roughly proportional to the degree of agonistic engagement of the individuals on the introduction days. There was also an increase in the standard errors of the post introduction means which reflected the variability of the recovery process across days. Full recovery of stable performance took 7-10 days in these monkeys. Because of its sensitivity to social manipulations, the use of operant schedules of this type appears to be of considerable value as a behavioral indicator of social stress and might be considered as an adjunct to the hormonal measures in future work.

Table 10

Total Responses (Means +/- SEM across days) on Both Components of a RI RI 1-Min Reinforcement Schedule During Social Manipulations. I-Troop, Summer, 1988.

ANIMAL:	QUOTATION	YUK	YAMAMOTO	EQUAL
Preformation (3 days)	188.7 (15.3)	3054.0 (445.0)	2945.3 (143.6)	953.0 (34.5)
Group Formed (1 day)	296	3535	4182	1010
Rhetoric In (1 day)	183	704	1833	861
Post Rhetoric (4 days)	360 (75.1)	2552.7 (566.0)	4105.0 (195.4)	901.3 (31.8)
Pre Lucifer (3 days)	922 (25.2)	3705.3 (233.5)	6868.0 (52.3)	880.0 (180.3)
Lucifer In (1 day)	830	2125	626	215
Post Lucifer (4 days)	240.0 (59.8)	3644.8 (498.1)	5687.8 (582.0)	486.3 (44.2)
Horatio In (1 day)	87	172	41	169
Post Horatio (4 days)	229.8 (40.6)	2156.8 (786.5)	2111.8 (624.9)	374.3 (64.1)

The results of the assays for serum PRL and cortisol are summarized in Table 11. Quotation's PRL values were low on the group reformation day, but he interacted very little with the other members of the group and it does not appear that he was under much stress; his relatively small increase in cortisol is only about half that seen in the other three monkeys. Rhetoric's data are more puzzling since neither hormone showed an increase when he was introduced and he was harassed constantly by the other monkeys. He also was aggressed against by Equal and Yamamoto on the day Lucifer was introduced and showed no increase in PRL on that day. There was a considerable delay in obtaining his blood sample each day because he refused to enter the laboratory with the other monkeys after his introduction and we would have to go into the compound and retrieve him after the other animals had been removed. Nevertheless, the lack of any detectable response is unusual.

Conceivably, his PRL was depleted, by the stress of the social situation which he did not adapt to at all well - he exhibited very little affiliative behavor for weeks following his introduction. Horatio and Lucifer also had reduced PRL values on the day of introduction which tends to support this idea. However, their cortisol values were elevated on the introduction day while Rhetoric's were not. Horatio, who achieved better integration into the group than Rhetoric, had relatively high PRL levels during the two weeks following the day of his introduction. The PRL data are quite variable and there are no obvious correlations with the mitogen SI's. Both this study and the I-Troop shock data described earlier suggest the hypothesis that the PRL response to stress in these monkeys is biphasic. An initial increase in PRL to a stressor may be followed by a decrease if the stressor is sufficiently intense, persists a long time, or is presented repeatedly. In the proposal for continuation we have proposed two parametric footshock experiments involving repeated sampling within and across shock sessions to define the course of the PRL response to stress. Once such informmation is in hand, it will be applied to the design of future experiments on social stress.

The mean cortisol values for I-Troop were significantly elevated on the day of group reformation and on the days when Lucifer and Horatio were introduced. Once again, the Rhetoric introduction proved an exception to the general pattern.

The objective of the initial experiment involving I-Troop was to determine whether or not manipulations of the social group which were designed to produce stress would result in changes in immune system responses and to get some idea of the nature and direction of such changes. The SI data indicated that changes were occurring and that they involved interactions between initial PBL levels and the responses to stimulation by the mitogens used; the results were persistent and biphasic. Both the reductions in bar pressing on the operant task and the increases in serum cortisol indicated that the social manipulations were stressful; however, the PRL data were not consistent with the idea that PRL levels invariably increase under stress and suggested the presence of a biphasic response that needs further investigation. Intense stress may deplete PRL or it may produce an inhibition of PRL release, probably by a dopaminergic mechanism. In the case of repeated or chronic stress, secretion of PRL may become habituated to the stressor. An understanding of the processes underlying the PRL data obtained in the work with I-Troop will be required before we can elucidate the PRL-immune function interactions in which we are interested.

Table 11

Prolactin and Cortisol Values in I-Troop, June-July 1988. Data for Animals not yet in Group are in Parentheses.

Prolactin (ng/ml):

Date:	6/17-6/20 (2 days)	6/24 Group Reformed	6/27 Rhetoric Intro	7/1-7/15 (3 days)
Animal:				
Equal	(5.1)	7.8	5.0	11.4
Yuk	(5.4)	15.1	6.1	11.5
Yamamoto	(14.7)	39.6	19.5	23.9
Quotation	(5.8)	5.9	19.4	33.4
Rhetoric	(3.6)	(no sample)	3.0	6.5
Lucifer	(4.6)	(4.3)	(no sample)	(10.8)

Cortisol (ug/100ml):

Equal	(39.4)	>75.0	41.8	42.5
Yuk	(42.7)	63.0	44.9	35.0
Yamamoto	(55.5)	72.6	38.5	52.7
Quotation	(37.6)	49.1	34.2	57.5
Rhetoric	(31.9)	(35.3)	36.3	26.
Lucifer	(47.3)	(45.5)	(32.5)	(51.9)

Prolactin (ng/ml):

Date:	7/18 Lucifer Intro Yuk Out	7/22 (1 day)	7/25 Horatio Intro;	7/29-8/12 (5 days)
Animal:				
Equal	21.7	10.9	22.5	18.9
Yuk	(no sample)	3.3	4.7	2.9
Yamamoto	21.2	17.0	4.8	10.9
Quotation	66.4	no sample	lost sample	22.4
Rhetoric	4.0	4.3	3.4	4.3
Lucifer	6.4	(dead)	-	-
Horatio		(17.4)	4.6	38.7

Cortisol (ug/100ml):

Equal	58.1	56.6	51.7	47.9
Yuk	(no sample)	(lost sample)	56.6	32.5
Yamamoto	66.2	>75.0	71.1	47.5
Quotation	52.6	no sample	60.7	34.4
Rhetoric	58.4	44.1	64.5	38.5
Lucifer	66.9	(dead)	-	-
Horatio		(33.8)	>75.0	42.9

2. <u>NT-Troop</u>:

The purpose behind the study of social behavior and immune function in NT-Troop (corresponding to Experiments 4 and 5 in the original proposal) was to look at what might happen in a large group of monkeys as a function of the manipulation of the social environment of the troop. The PBL responses to mitogen stimulation were monitored in a total of 14 members of NT-Troop during the study. Two nonlactating adult females, one subadult female, one juvenile female and a juvenile male were removed from NT-Troop and given 4 weeks adaptation to the handling and blood collection procedures prior to their reintroduction to the troop on August 17, 1988. Six of the eight oldest adult males also were monitored, as were a subadult male (formerly a member of NT-Troop) and two adult males from C-Troop. The last three monkeys were introduced as "strangers" during the course of the study. In addition, one of the six adult males had to be removed from the troop for two weeks for treatment of an injury; his reintroduction was treated as a social manipulation.

Daily social behavior observations took place prior to blood collection on the days the animals were sampled. Except for the return of the females and juveniles which was scheduled two days after the blood collection for baseline, the social manipulations were scheduled to coincide with the collection days. Social behavior was scored as for the I-Troop study; scores were obtained for 23 monkeys, including the 14 participating in mitogen response monitoring as well as 9 other adult males and females. The 24th slot (see section A.2.b.) was used to collect data on all of the remaining monkeys in the troop that were not being scored individually. During the introductions, two observers were used to collect data; one used a scan procedure while the other did focal observations on the 14 experimental monkeys.

In terms of the schedule employed, the baseline blood collection for the mitogen assays was done on 8/15/99; the 5 females and juveniles were returned to the troop on 8/17; blood was collected on 8/19, on 8/22 when Patek, a C-Troop male, was introduced and on 8/26 and 8/29. Following a two week break, samples were obtained twice a week beginning on 9/12 and continuing through 9/30 after which weekly samples were taken until the end of the project. Rasputin, another C-Troop male, was introduced on 9/23 and Alien, the young male that had been removed from NT-Troop at the end of February, was returned on 9/27. Ouezel, an adult malee participant in the mitogen study group was removed on 9/14 for treatment of bite wounds and returned on 9/29. Achmed, the juvenile male, was removed for treatment for diarrhea on 10/4 and returned on 10/11. These events, as well as others occurring during this period, are

listed in Table 12 which also provides the key to the figures on the following pages.

Figure 10 gives the individual SIs to ConA (MTT assay procedure) for the entire experiment with the events listed in Table 12 keyed below the date on which that event occurred; Figure 11 gives the means (+/- SEM) for the group. Figures 12-14 contain the SIs to CcnA for selected sets of individuals and Figure 15 provides similar information for the five monkeys introduced to, or returned to the group following removals for varying numbers of days. In the cases of Rasputin and Alien, we obtained before and after blood samples on the day they were introduced. Their data for the introduction and subsequent days are given in Figure 16.

In the case of the introductions and reintroductions, all five females and juveniles reintroduced on 8/17, had lower SI's to ConA on 8/19 than they did on 8/15. With the exception of Patek, who did not change, the other four males that were introduced or reintroduced also showed lower SI's. Typically, the decreases in SI's were followed by a rebound in which there was an overshoot of the preintroduction SI.

With only a few exceptions on a few days, SI's to ConA in the regular members of the troop were quite stable. The major fluctuations involved general increases in SI's three days after Patek's introduction and right after the introductions of Rasputin and Alien on 9/23 and 9/27. These large excursions in the curves are brief and are followed by a rapid return to baseline. Dahlia and Kilgore, the two young females, did not exhibit these kinds of changes while the two adult females, Lilly and Dusty did. Achmed, the juvenile male, exhibited the adult pattern, showing a depression on 9/23, a strong rebound on 9/27 followed by return to baseline during the next week. As in the case of I-Troop, there was the suggestion of a cyclicity; it is most apparent in the data of the monkeys that did reacting very much to the social manipulations but can be seen in the others as well. The large increase in Lilly's SI on 11/1 is an anomaly for wich we have no ready explanation.

Stimulation indexes to PHA are summarized in Figure 17. The mean SI for NT-Troop's response to PHA decreased with the introduction of Patek and Rasputin but increased after Alien's introduction. This pattern is comparable to that observed for ConA, but with the large variation in PHA SI's on control days, the correlations between social variables and the responses to PHA stimulation were not significant. As there were no consistent changes across days or social manipulations, the data have been plotted only through 9/30; however, PHA assays of the NT-Troop samples were continued throughout the duration of the project.

TABLE 12: SUMMARY OF 14 INDIVIDUAL MONKEYS OF NT TROOP BY SOCIAL EVENTS

Event	Date	Summary Of Social Activity
--	8/15/88	First Mitogen Assay
A-Reintroduction	8/17/88	Duster, Dahlia, Kilgore, Lilly and Achmed returned to troop
B-Introduction	8/22/88	Patek introduction, cuts; more cuts 8/25 Kukla and Dusty cuts on 8/26
C	9/6/88	Weed removed for treatment of diarrhea
D	9/12/88	Weed returned
E	9/14/88	Ouezel out to treat cuts
F	9/16/88	Weed cuts
G-Introduction	9/23/88	Rasputin introduction. PBL before and after. Cuts; 9/26 Rasputin and Hobbit cuts
H-Introduction	9/27/88	Alien introduction
I-Reintroduction	9/29/88	Ouezel returned
J	10/4/88	Hobbit, Rasputin, Patek being treated for cuts. Achmed removed for treatment of diarrhea
K-Reintroduction	10/11/88	Achmed returned
L	10/14/88	Ouezel cuts on shoulder
M	10/17/88	Hobbit cuts on ear
N	10/24/88	Hobbit cut on neck, Patek cuts
O	11/15/88	Rasputin removal for cuts (permanent removal)
P	11/29/88	Kuhla, Barker and Hobbit treated for cuts
Q	11/30/88	Kukla removed for sutures

SOCIAL–INDUCED ALTERATION of LYMPHOCYTES REACTIVITY for Con A during GROUP MANIPULATION of N T TROOP (AUGUST–DECEMBER 1988)

(SUMMARY OF 14 MEMBERS)

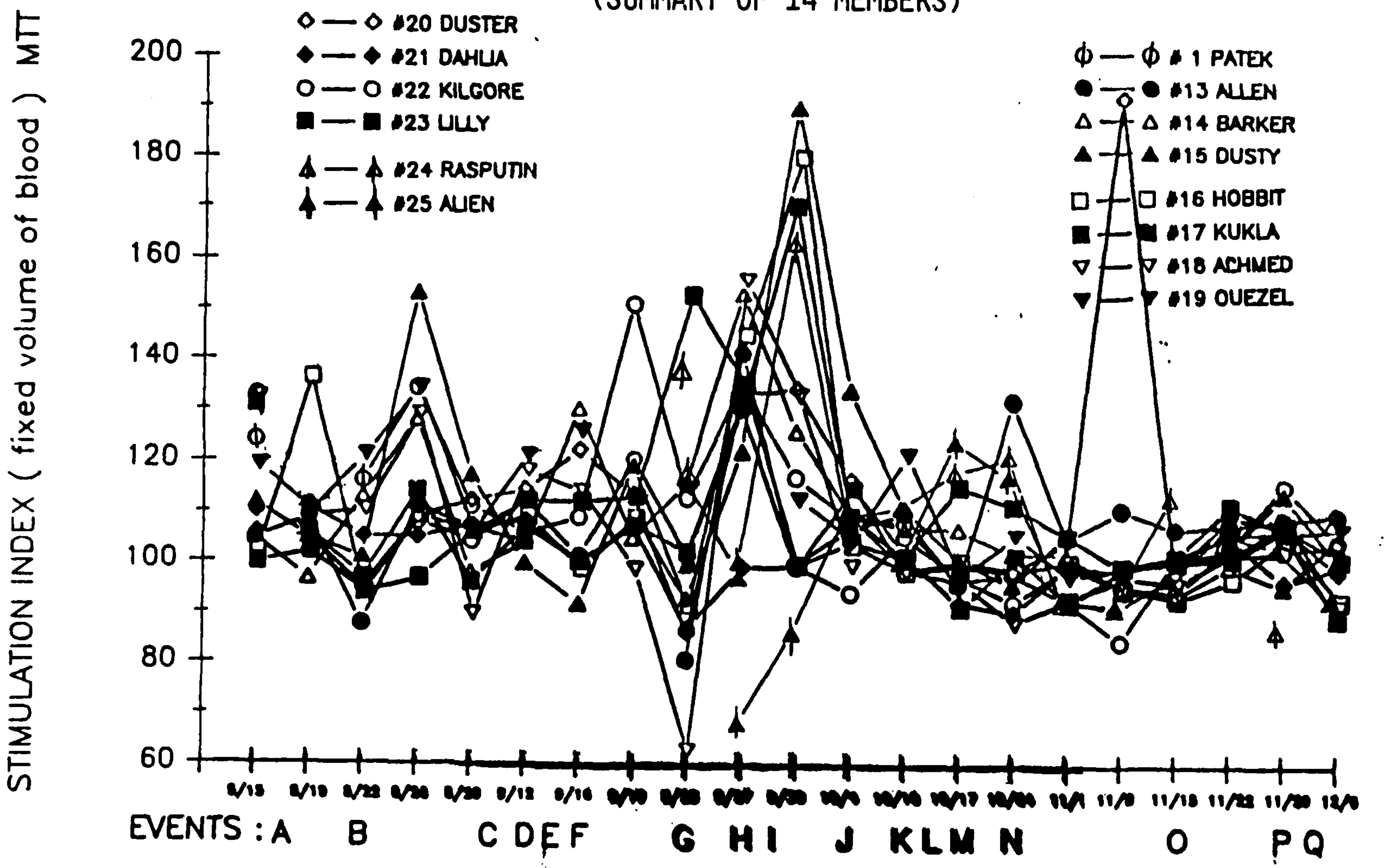


FIG. 10: SUMMARY OF INDIVIDUAL MONKEY PBL CON A RESPONSE DURING SOCIAL MANIPULATION OF NT TROOP (SEE TABLE 12 FOR DESCRIPTION OF SOCIAL EVENT)

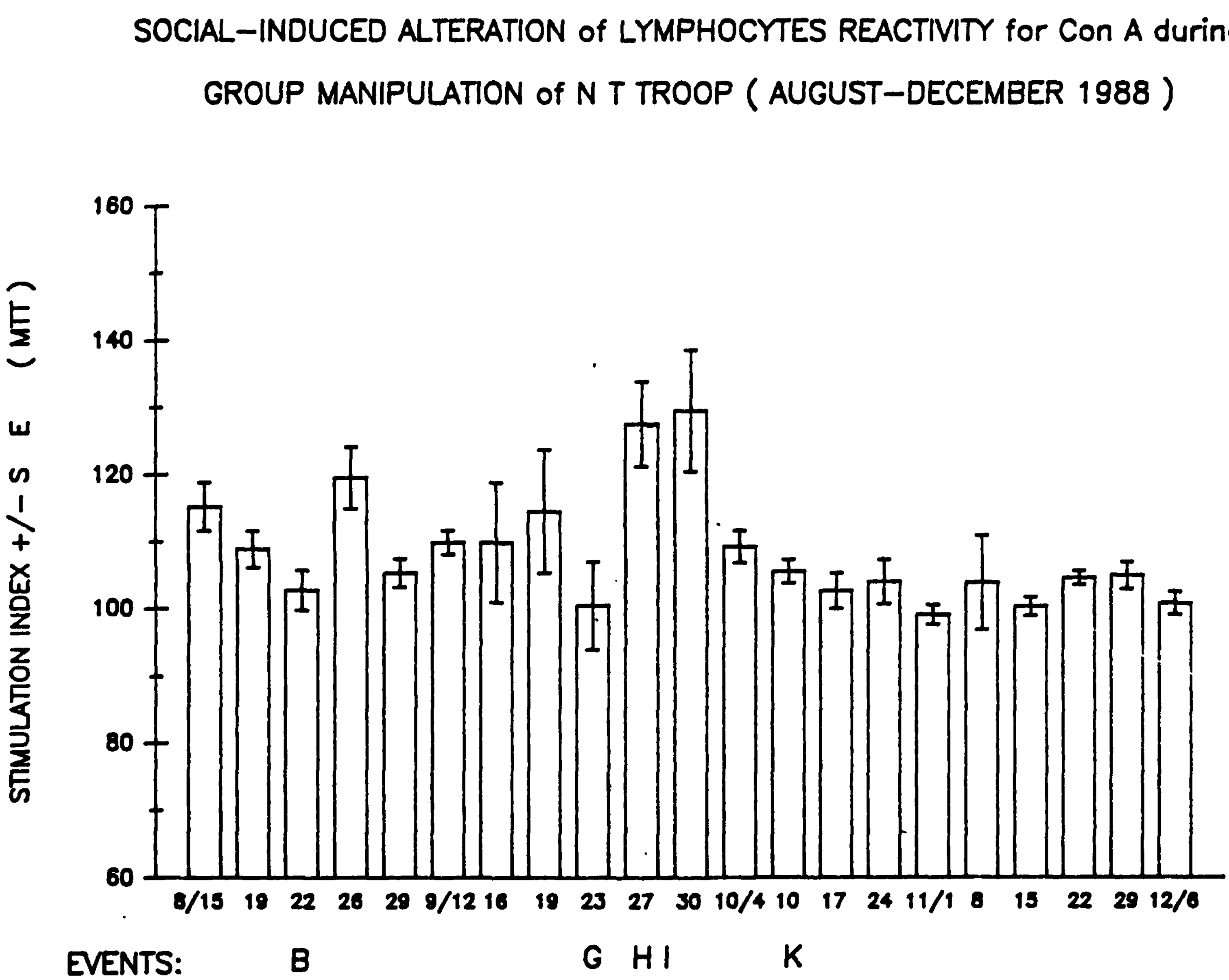


FIG. 11: MEANS OF NT TROOP PBL CON A REACTIVITY DURING SOCIAL GROUP MANIPULATION

SOCIAL–INDUCED MANIPULATION of LYMPHOCYTE REACTIVITY for Con A during GROUP MANIPULATION of N T TROOP (AUGUST–DECEMBER 1988)

(SUMMARY OF ♂ MEMBERS)

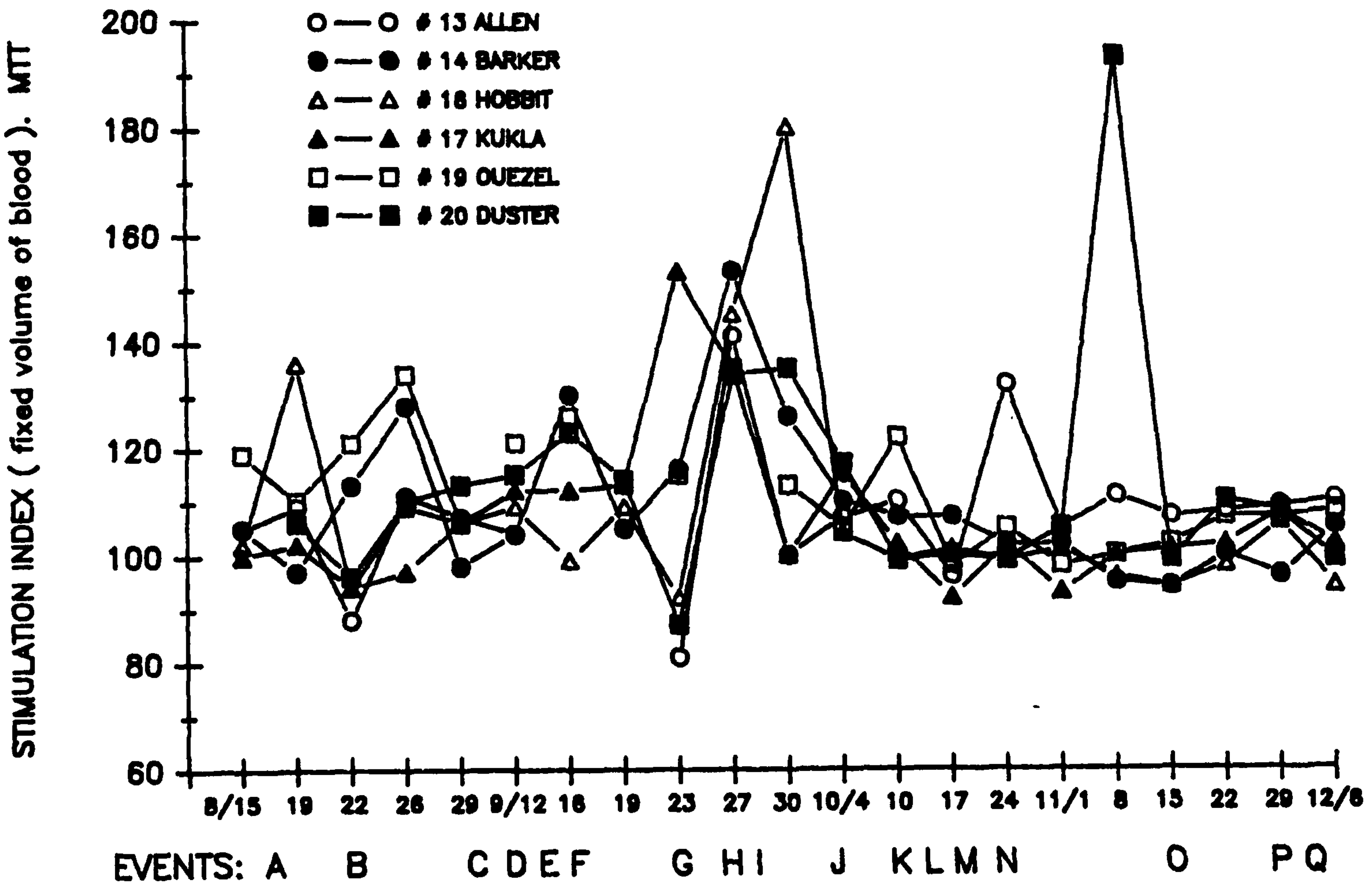


FIG. 12: INDIVIDUAL MALE MONKEY PBL CON A RESPONSE DURING SOCIAL MANIPULATION OF NT TROOP

SOCIAL–INDUCED MANIPULATION of LYMPHOCYTE REACTIVITY for Con A during GROUP MANIPULATION of N T TROOP (AUGUST–DECEMBER 1988)

(SUMMARY OF ♀ and young MEMBERS)

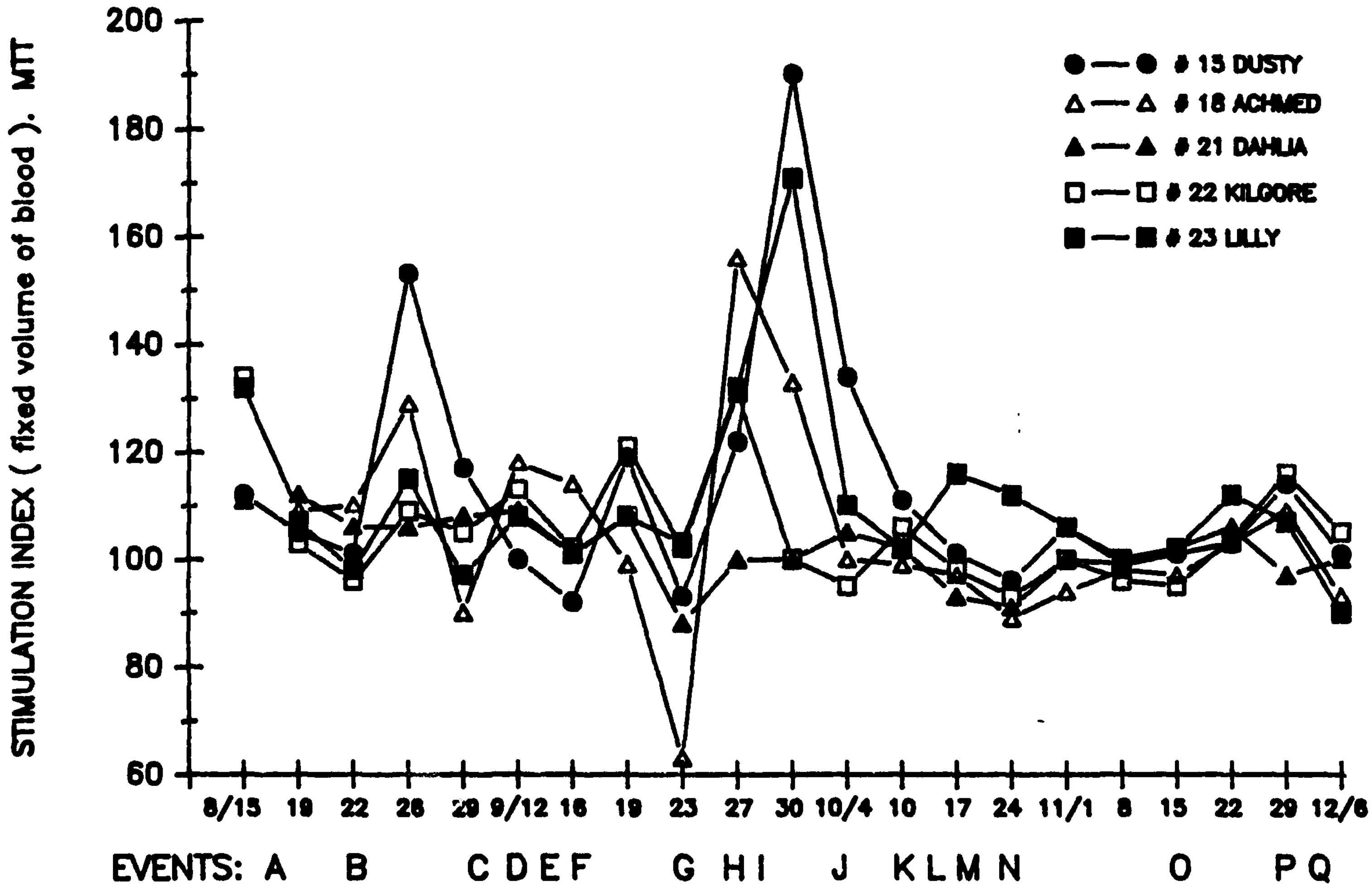


FIG. 13: INDIVIDUAL FEMALE OR YOUNG MONKEYS PBL CON A RESPONSE DURING SOCIAL MANIPULATION OF NT TROOP

SOCIAL-INDUCED ALTERATION of LYMPHOCYTES REACTIVITY for Con A during GROUP MANIPULATION of N T TROOP (AUGUST-DECEMBER 1988)

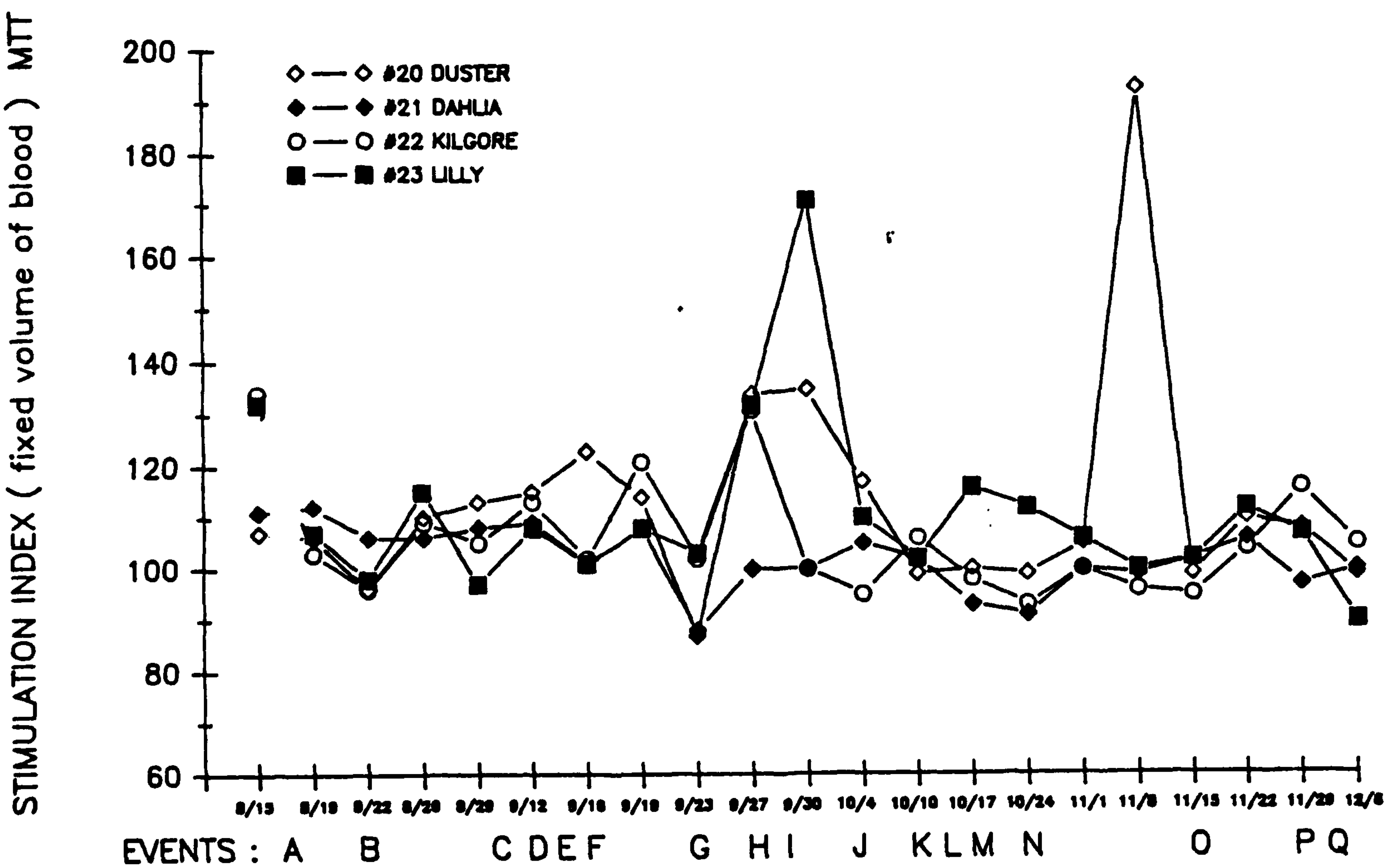


FIG. 14: PBL CON A RESPONSE OF INDIVIDUAL MONKEYS DURING REFORMATION OF NT TROOP

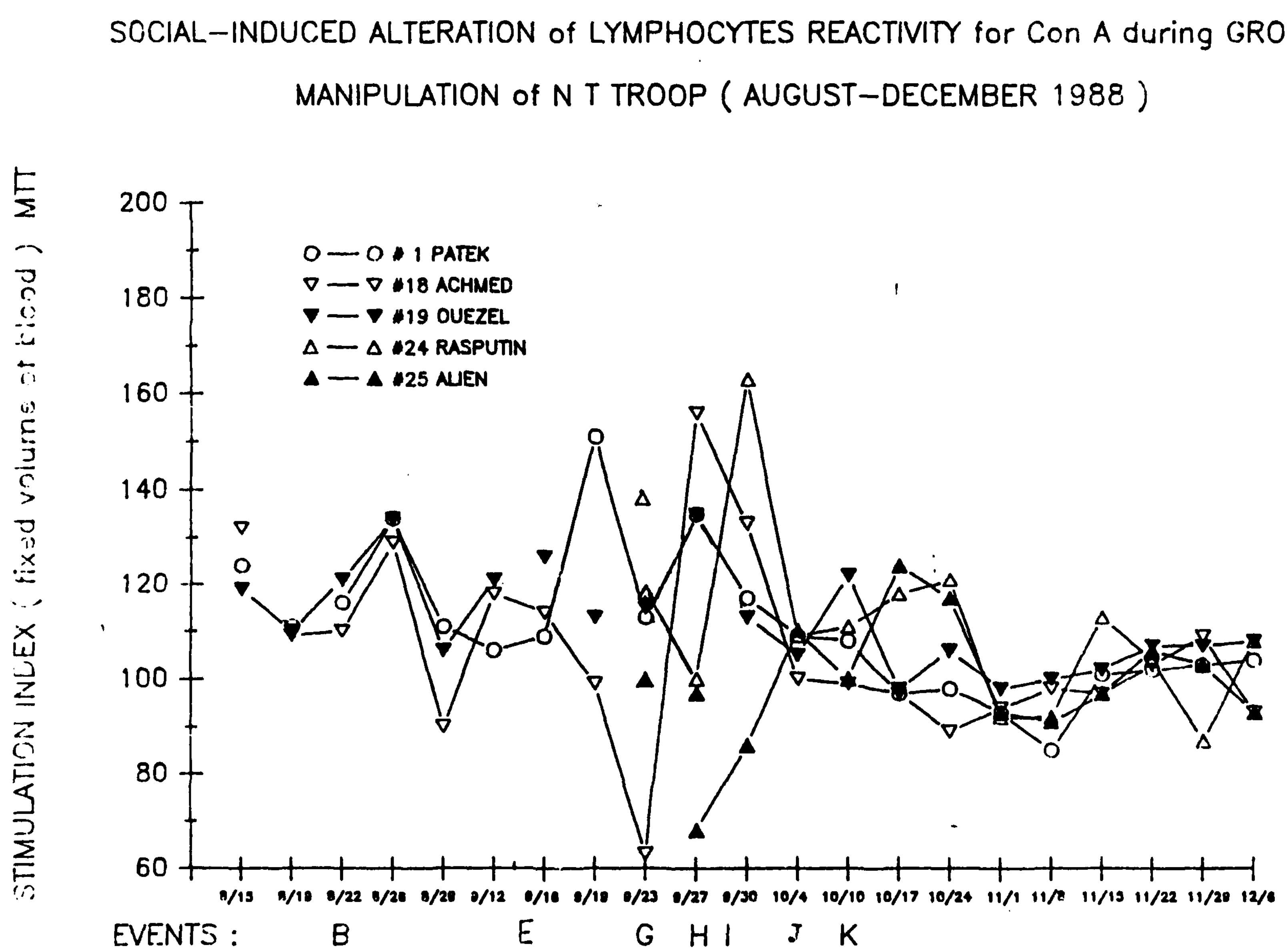
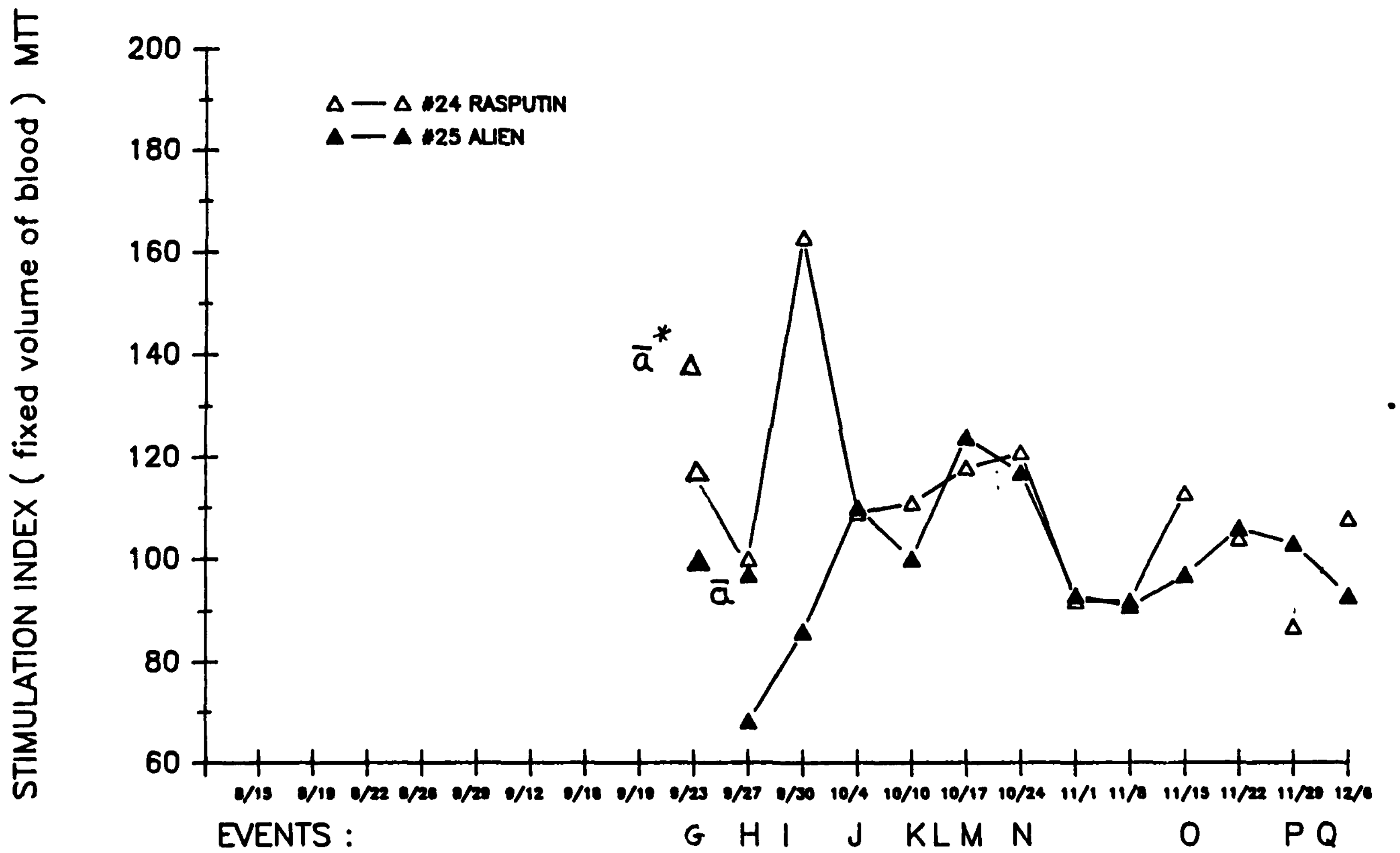


FIG. 15: PBL CON A RESPONSE OF INDIVIDUAL MONKEYS UPON INTRODUCTION OR REINTRODUCTION INTO NT TROOP (SEE TABLE 12 FOR DESCRIPTION OF EVENTS)

SOCIAL-INDUCED ALTERATION of LYMPHOCYTES REACTIVITY for Con A during GROUP MANIPULATION of N T TROOP (AUGUST-DECEMBER 1988)



a* before introduction

FIG. 16: PBL CON A REACTIVITY IMMEDIATELY BEFORE AND AFTER INTRODUCTION INTO NT TROOP

SOCIAL–INDUCED ALTERATION of LYMPHOCYTE REACTIVITY for PHA during GROUP MANIPULATION of N T TROOP (AUGUST–SEPTEMBER 1988)

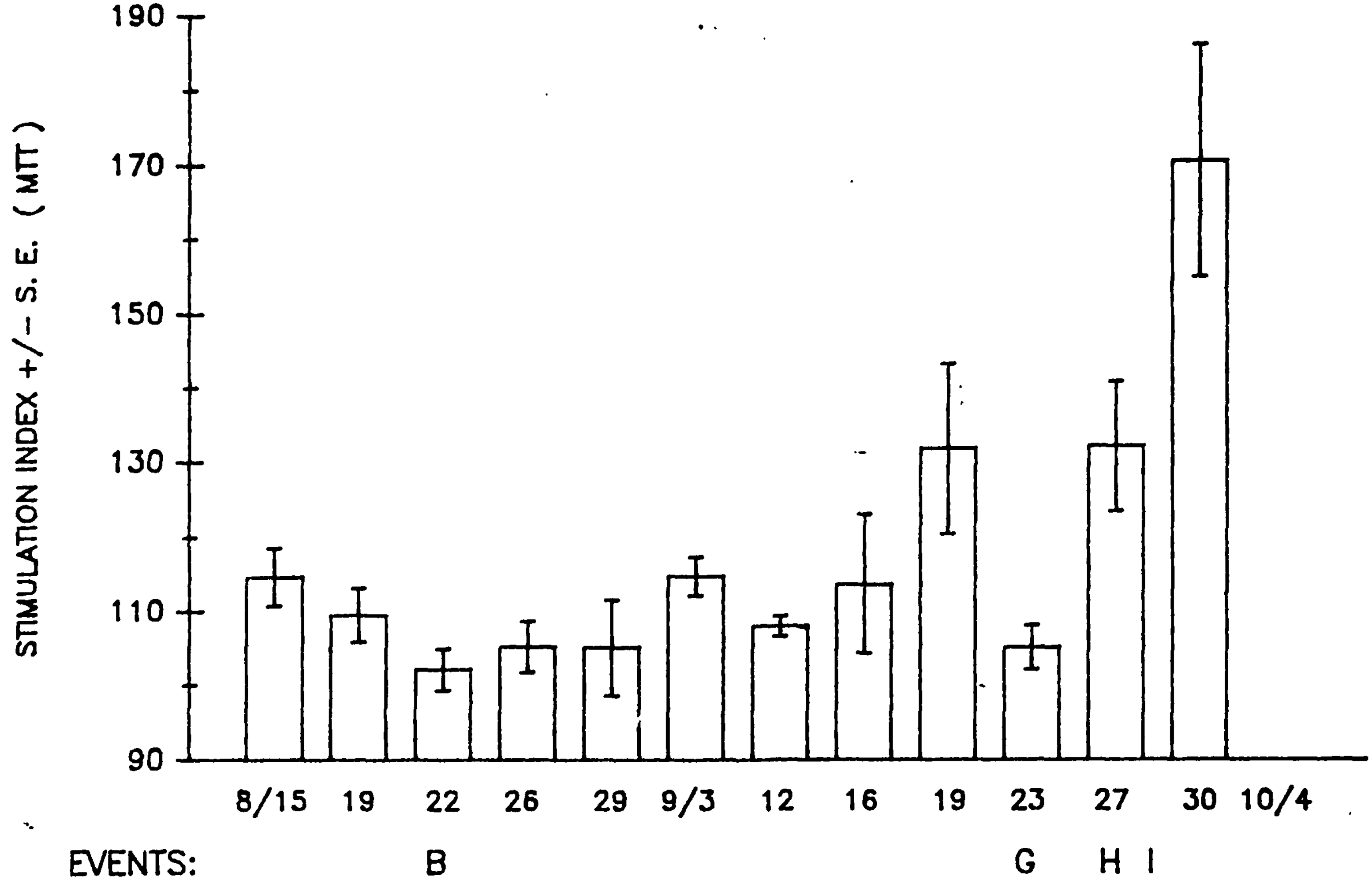


FIG. 17: MEANS OF NT TROOP PBL-PHA REACTIVITY DURING SOCIAL GROUP MANIPULATION

Table 13

Submissive, Aggressive and Affiliative Behavior in NT-Troop August - November 1988. Data are given as mean frequencies per animal per day.

	Date					
	8/15-17	8/18-22 Juveniles, Females In	8/22 Patek Intro	9/6-15	9/20-23 Ouezel Out	9/23 Rasputin Intro
Days of Data:	(3)	(5)	(1)	(3)	(4)	(1)
Submission	1.6	1.5	3.2	0.9	1.8	3.0
Aggression	1.7	1.4	4.9	0.7	2.1	8.4
Affiliative	5.3	4.7	1.7	2.6	5.6	0.2
	9/24-27	9/27 Alien Intro	9/29 Ouezel Reintro	10/3-7	10/10-14	10/17-30
Days of Data:	(4)	(1)	(1)	(5)	(4)	(9)
Submission	1.0	0.4	2.7	0.9	0.9	0.4
Aggression	1.4	0.5	4.2	1.0	0.7	0.4
Affiliative	3.4	1.3	4.2	5.6	6.6	3.3

Social behavior was analyzed in blocks of 3 or 4 days for comparison to the mitogen stimulation data. Days on which introductions took place were analyzed separately to determine the acute effects of the introductions on social behavior. Frequencies of submissive, aggressive, and affiliative behaviors within the group are given in Table 13.

The most striking thing about the social data was the consistent reduction in affiliative behavior which occured with each introduction or reintroduction. The introduction of the two strangers, Patek and Rasputin, produced increases in agonistic behavior which primarily involved the two strangers. The reintroduction of the young male, Alien, produced little agonistic behavior in the group; Ouezel's reintroduction produced a lot throughout the troop, although much of it involved Patek, Rasputin and Alien and not Ouezel. The introduction of strangers provokes aggression against the strangers. As has been reported before (Bunnell and Iturrian, 1988), the reintroduction of a familiar member of the male hierarchy produces widespread agonistic behavior that does not necessarily involve the animal being returned to the group. It is as if the latter situation engenders a testing of existing social bonds and relationships.

There were no significant correlations between ConA or PHA SI's and individual scores on any of the three categories of behavior.

The prolactin data obtained during the social manipulations of NT-Troop are summarized in Table 14.

Table 14

Prolactin Values During Social Manipulations in NT-Troop: August - November 1988. Data for Animals not yet in Group are in Parentheses. (Achmed and Dahlia were juveniles; Kilgore was a subadult; remainder were adults.)

Prolactin (ng/ml):

Date:	8/15	8/19 Juveniles, Females In on 8/17	8/22 Patek Intro	8/26	8/29	9/19 Ouezel Out	9/23 Rasputin Intro
Animal:							
Males:							
Allen	8.4	5.3	2.8	2.8	3.6	2.2	3.1
Barker	20.7	6.9	9.8	12.2	6.1	15.3	12.0
Hobbit	17.8	5.2	2.7	9.7	12.2	3.1	5.8
Kukla	49.3	49.5	10.2	4.6	13.7	5.5	6.0
Ouezel	14.6	4.4	2.6	6.9	17.5	(7.5)	(4.7)
Duster	33.8	6.9	2.9	2.2	13.9	2.4	2.8
Achmed	(2.0)	no sample	1.0	no sample	2.8	2.4	3.0
Patek	(34.1)	(3.1)	4.4	2.0	3.3	3.0	3.4
Rasputin							(7.8);17.5
Alien							(19.2)
Females							
Dusty	(15.4)	5.5	8.3	5.2	4.3	3.4	4.6
Lilly	(12.3)	15.7	2.8	4.1	4.6	13.2	17.5
Kilgore	(33.8)	7.0	5.4	13.5	21.5	15.4	14.6
Dahlia	(2.3)	2.2	1.9	2.3	4.5	1.9	3.4

Date:	9/27 Alien Intro	9/30 Ouezel In 9/29	10/04	10/10	10/17	10/24	11/01
Animal:							
Males:							
Allen	3.0	2.2	2.3	2.1	2.4	2.5	2.2
Barker	13.8	5.6	5.8	5.2	7.8	5.3	5.2
Hobbit	14.4	3.2	2.5	2.5	2.9	2.9	1.9
Kukla	3.7	1.8	3.6	3.4	3.4	2.8	1.9
Ouezel	(11.3)	2.6	3.9	5.5	6.5	2.3	2.4
Duster	3.4	1.8	1.8	3.2	2.2	1.9	2.2
Achmed	2.6	1.9	no sample	2.7	3.0	2.6	no sample
Patek	3.2	2.5	3.1	14.3	19.8	3.5	2.6
Rasputin	3.7	3.0	4.8	4.1	3.9	7.4	2.9
Alien	2.8	5.4	19.5	7.8	35.9	25.1	5.1
Females							
Dusty	7.6	5.4	4.5	3.7	2.6	2.9	2.5
Lilly	51.4	22.4	>100.0	>100.0	43.4	>100.0	>100.0
Kilgore	7.8	4.5	4.3	20.9	12.1	5.1	3.6
Dahlia	2.9	2.0	2.2	2.8	2.6	2.4	2.7

PRL levels were high on 8/15; this probably reflects an incomplete readaptation the restraint/blood collection procedures. There was considerable variation in individual values throughout the course of study and there were no correlations between PRL scores and either SI's or social behavior. The large number of animals sampled each time blood was collected and the accompanying delay between social testing and blood collection undoubtedly accounts for the failure to find any significant relationships. The samples from Lilly, who showed very high levels of PRL during October were reassayed with the same results; we have no explanation for her high values and, to the best of our knowledge, she was not pregnant at this time.

The cortisol data obtained during the social manipulations of NT-Troop are summarized in Table 15:

Table 15

Cortisol Values During Social Manipulations in NT-Troop: August - November 1988. Data for Animals not yet in Group are in Parentheses. (Achmed and Dahlia were juveniles; Kilgore was a subadult; remainder were adults.)

Cortisol (ug/100ml):

Date:	8/15	8/19 Juveniles, Females In on 8/17	8/22 Patek Intro	8/26	8/29	9/19 Ouezel Out	9/23 Rasputin Intro
Animal:							
Males:							
Allen	22.2	24.8	26.8		46.7	44.9	36.1
Barker	28.9	27.8	30.9		65.4	56.7	64.0
Hobbit	23.1	27.0	34.8		64.4	55.4	41.8
Kukla	23.0	24.6	27.1		61.6	55.7	43.9
Ouezel	27.3	32.9	32.0		68.2	(57.6)	no sample
Duster	20.3	24.4	27.5		54.5	62.4	52.5
Achmed	(28.6)	no sample	24.1	no sample	>75.0	>75.0	>75.0
Patek	(30.2)	(29.2)	37.6		47.6	51.6	>75.0
Rasputin							(29.9);71.2
Alien							(44.8)
Females							
Dusty	(31.6)	58.4	43.8		>75.0	>75.0	>75.0
Lilly	(24.8)	29.1	27.2		no sample	53.4	38.6
Kilgore	(35.0)	>75.0	64.0		>75.0	71.4	68.6
Dahlia	(35.1)	60.1	62.4		>75.0	69.6	>75.0

Table 15 (continued)

Date:	9/27 Alien Intro	9/30 Ouezel In 9/29	10/04 Achmed Out	10/10 Achmed In	10/17 Hobbit Cuts	10/24 Hobbit, Patek Cuts	11/01
Animal:							
Males:							
Allen	33.6	33.1	32.2	40.6	38.9	35.5	43.3
Barker	>75.0	51.0	49.0	53.6	45.8	57.0	>75.0
Hobbit	38.4	30.8	32.2	36.7	44.6	35.4	61.8
Kukla	48.6	44.4	51.8	58.9	51.6	62.8	62.4
Ouezel	(no sample)	58.1	47.1	59.7	66.3	62.6	72.8
Duster	43.5	43.8	67.7	49.7	62.8	69.8	70.9
Achmed	>75.0	>75.0	no sample	61.8	>75.0	>75.0	no sample
Patek	>75.0	53.9	45.3	53.0	57.2	63.6	62.6
Rasputin	39.0	35.8	37.6	39.5	43.7	55.5	57.9
Alien	>75.0	66.4	59.3	62.8	48.5	49.3	73.3
Females							
Dusty	>75.0	61.3	74.3	72.4	>75.0	72.2	>75.0
Lilly	50.1	54.5	--	Insufficient sample		--	59.7
Kilgore	>75.0	54.6	72.7	66.8	72.7	>75.0	>75.0
Dahlia	>75.0	69.8	71.4	71.6	>75.0	>75.0	>75.0

The shift in the range of values for cortisol between 8/22 and 8/29 reflects the use of the different assay kits described earlier. Despite the length of time betwen social testing and obtaining blood samples from all 14 monkeys, the cortisol data were sensitive to social activity during the introductions. Correlations between SI's and cortisol values were nonsignificant.

A table of ACTH values for NT-Troop during the September introductions is given in Appendix B. There were no obvious relationships here beetween ACTH and Social behavior.

Figures 18 and 19 give the PRL and cortisol values which accompanied the introductions and reintroductions in both I- and NT Troops. Prolactin values increased in some monkeys and decreased in the others. There was no relationship between the direction of the changes in PRL on the day of the introduction and the changes in SI's observed. Cortical was elevated in all of the monkeys except Rhetoric, who also exhibited no increase in PRL (see the I-Troop discussion).

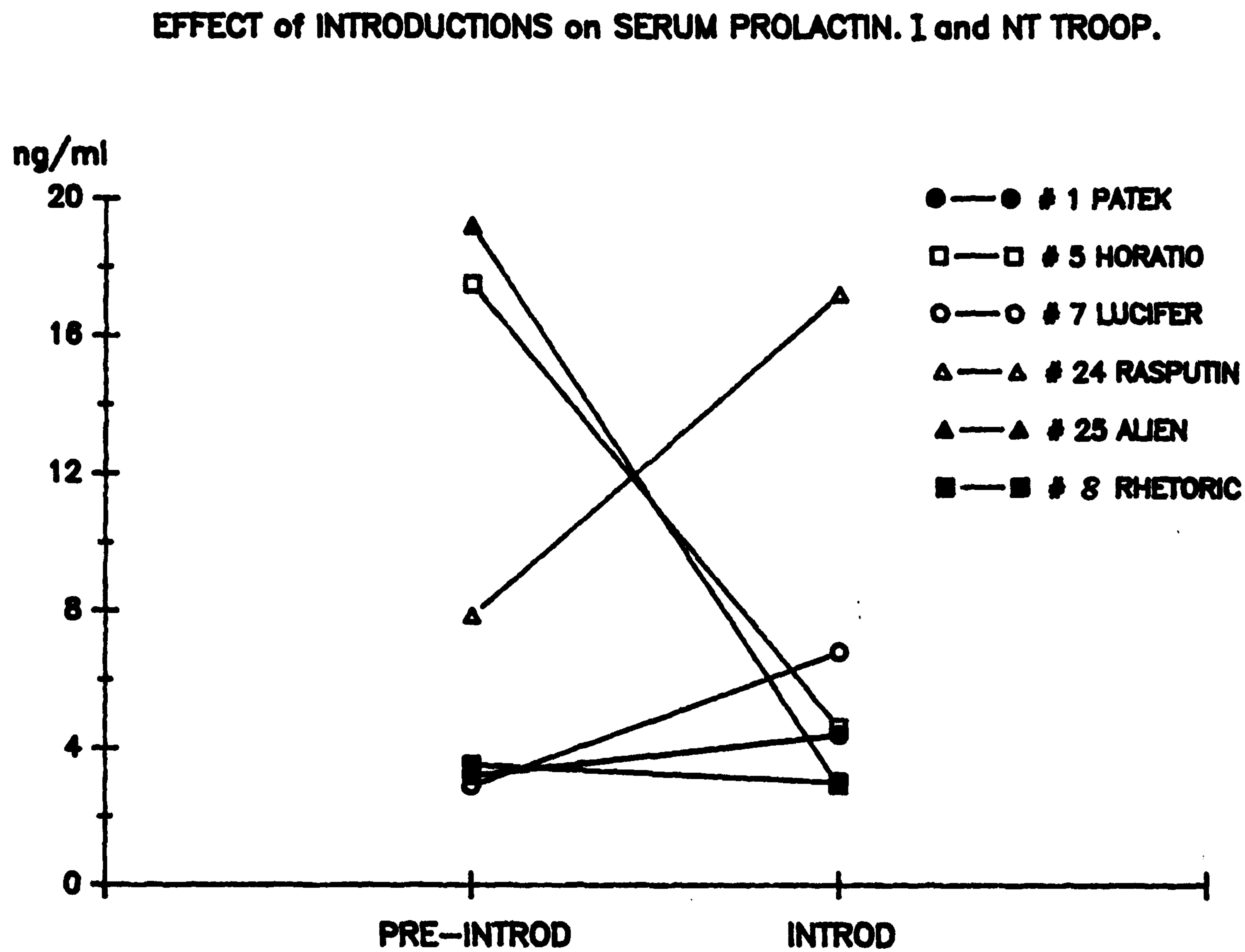


FIG. 18: EFFECT OF INTRODUCTION INTO I OR NT TROOP UPON SERUM PROLACTIN

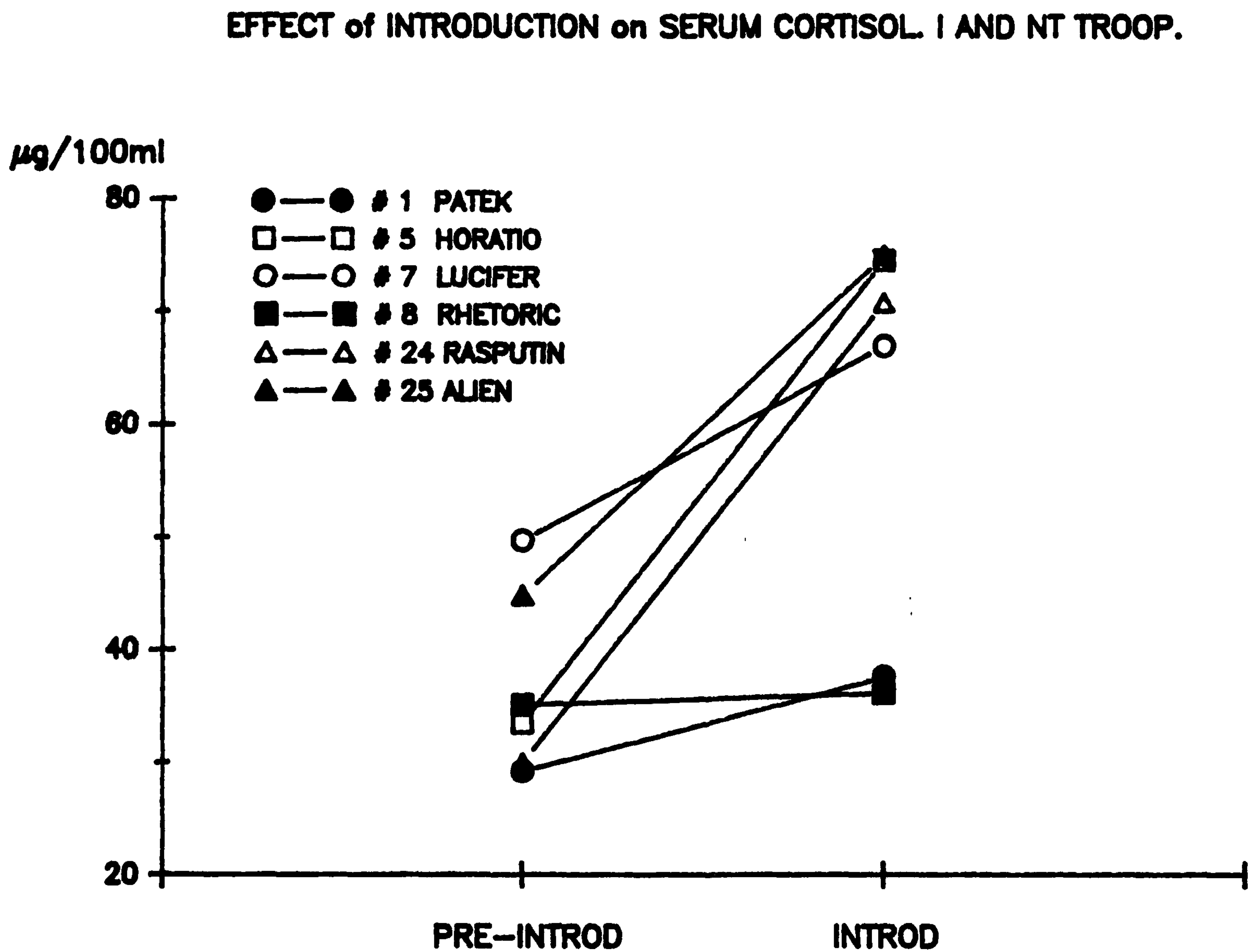


FIG. 19: EFFECT OF INTRODUCTION INTO I OR NT TROOP UPON SERUM CORTISOL

D. Pilot Work and Other Results.

In previous work (Bunnell & Iturrian, 1988) we had installed an operant panel, complete with cue light, manipulanda, and a pellet feeder in the indoor social test cage and examined performance on fixed ratio (FR) reinforcement schedules with 2-6 animals present in the cage. The presence of the panel produced a significant increase in the frequency of social interactions among the monkeys and, since only one animal could have access to the panel at a time, we were able to identify both agonistic and cooperative episodes as the animals exchanged places at the panel. During the present project, we installed a second operant panel next to the first and trained the six C-Troop males to perform on both panels. In the first pilot study with this arrangement, test sessions were divided into four intervals. During the first interval, both panels were activated; one was operational during the second interval after which it was turned off and the other activated for the third interval; both panels were on for the last interval. Initially the intervals were 5 min long; later the duration of the intervals was shortened to 2.5 min and the animals given two sets of 4 intervals during a test. To date, we have tested all possible pair combinations as well as groups of three animals and a five animal group. Schedule requirements have been increased from FR 10 to FR 30 and we have explored the procedure of using different schedule requirements on each of the two panels. Most of the social behavior observed during these test involved lipsmacking, hugging, and allogrooming, although two fights, both instigated by the same, intermediate ranked animal, took place during the first week of social observations. In the pair tests animals worked side by side with no agonistic episodes; with only one panel operating, animals would frequently displace other animals with little accompanying aggression. The assumption is that the animals being displaced are lower ranking than those doing the displacing. The two panel arrangement offers a number possibilities for producing competitive and cooperative behaviors. We are now ready to explore several protocols to determine their utility for producing or alleviating social stress. A number of these protocols will require the animals to earn a significant portion of their daily food ration by lever pressing. As indicated above, we have begun to examine the use of different schedule requirements on the two panels. We will be interested in seeing if the animals compete for the panel with the less stringent schedule requirements and in identifying the ways in which individuals of different social status cope with the situation behaviorally. In another test involving competition, both panels will be active; when one animal meets the schedule requirements it will be reinforced and the other animal will have its panel turned off before it meets criterion. Thus, the second monkey misses its

reinforcement on that trial and has to start over again following a brief time out. A third possibility is to have responses on one panel activate the feeder on the other panel - a situation which has the potential for generating both competitive and cooperative interactions.

In a related procedure, an operant panel was installed out of doors in the NT-Troop compound. The panel can be programmed by a built in computer to deliver a large range of FR reinforcement schedules. A light is used as a cue to indicate when food can be earned and the setup is made operational during selected social observation periods. Pilot work during the summer has been confined to observing the monkeys' responses to the panel and the acquisition of lever pressing by animals with no previous operant training (adult males that have had laboratory experience with operant schedules respond very quickly). We have had a number of apparatus failures with this setup. As most of these have been associated with difficulties in weatherproofing the computer and its interface circuitry, we plan to convert this operant station so that it can be run by our PDP 11/73 computer which is housed in the computer room in the laboratory. Extending the operant performance situation to an intact social group increases the potential for studying behavior in a more complex social situation than is provided by the indoor social testing with C-Troop. The long range objective of this procedure is the development of a "closed economy" social environment such that the members of one of our monkey troops will be required to earn their daily food rations which will be delivered according to operant schedules of reinforcement. There are a number of problems that have to be addressed before such a situation can be established, including determining the number of stations that would be required, the length of time the stations would have to be active each day, the amount of shaping of the response that will be required, and finding a means of automatically identifying each animal as it responds. A fairly extensive pilot project is proposed to determine the practicality of installing a closed economy in a social group. If it works, it would be a powerful tool for investigating coping behavior as a function of social variables and could be used in the study of social stress and immune function.

We have continued our studies of various methods of isolating T cells and devloping an optimal growth medium. Hamster sera (15%) was the only effective growth sera, but the control wells were 6-8 times higher than the PBL method so that SI's and changes in counts per minute (delta CPM) after mitogen stimulation were abnormally low. We also tried using the sera of a single monkey donor as a growth media additive for the mitogen assays. Although mitogen proliferative response was adequate, there was a poor correlation with the PBL data across individuals.

I-Troop has three individuals that usually have higher SI's than the others. Placing as little as 5% PBL from any of the high response individuals into the media elevated the SI and delta CPM for the mitogen assay. This suggests that high responsive inndividuals have a growth factor that is different from that of other individuals. No inhibition of proliferative response was produced by 15% PBL from low responders. I-Troop motogen response assayed by both the fixed initial cell number and fixed initial volume of PBL was compared across four weekly blood draws. The initial circulating PBL cell number varied for individuals during the month, but most values were in thhe 9,000 - 13,000 range.

The correlations between initial PBL number or volume and SI's were only .17 and .20, respectively. Constant cell number or constant volume produced different patterns of mitogen response. It is concluded the the clinical (constant volume) method is as valid as the constant initial cell number for studying mitogen responses in our monkey species. (Figures 20 and 21).

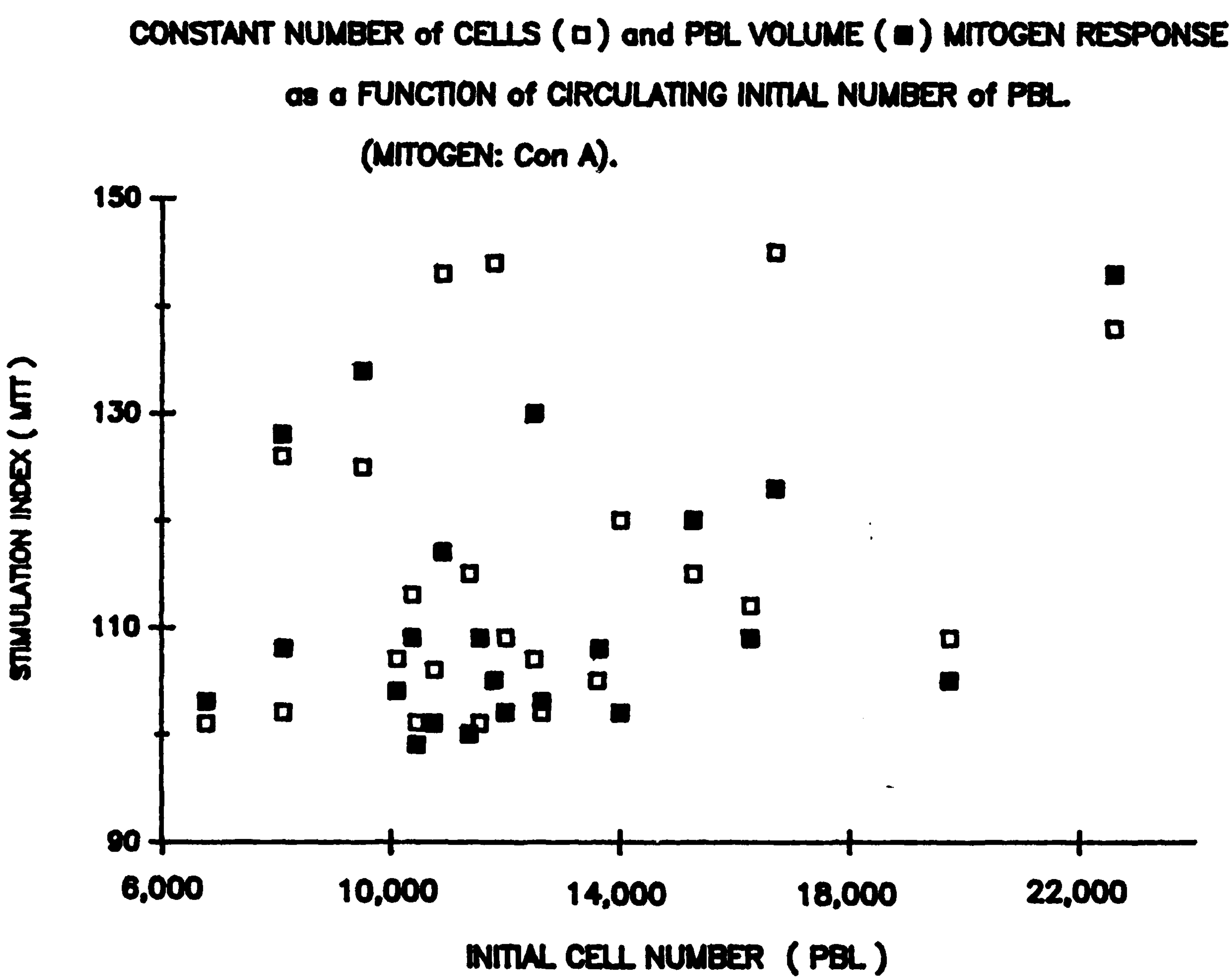


FIG. 20: COMPARISON OF METHODS OF EVALUATING CON A REACTIVITY AND NUMBER CIRCULATING PBL

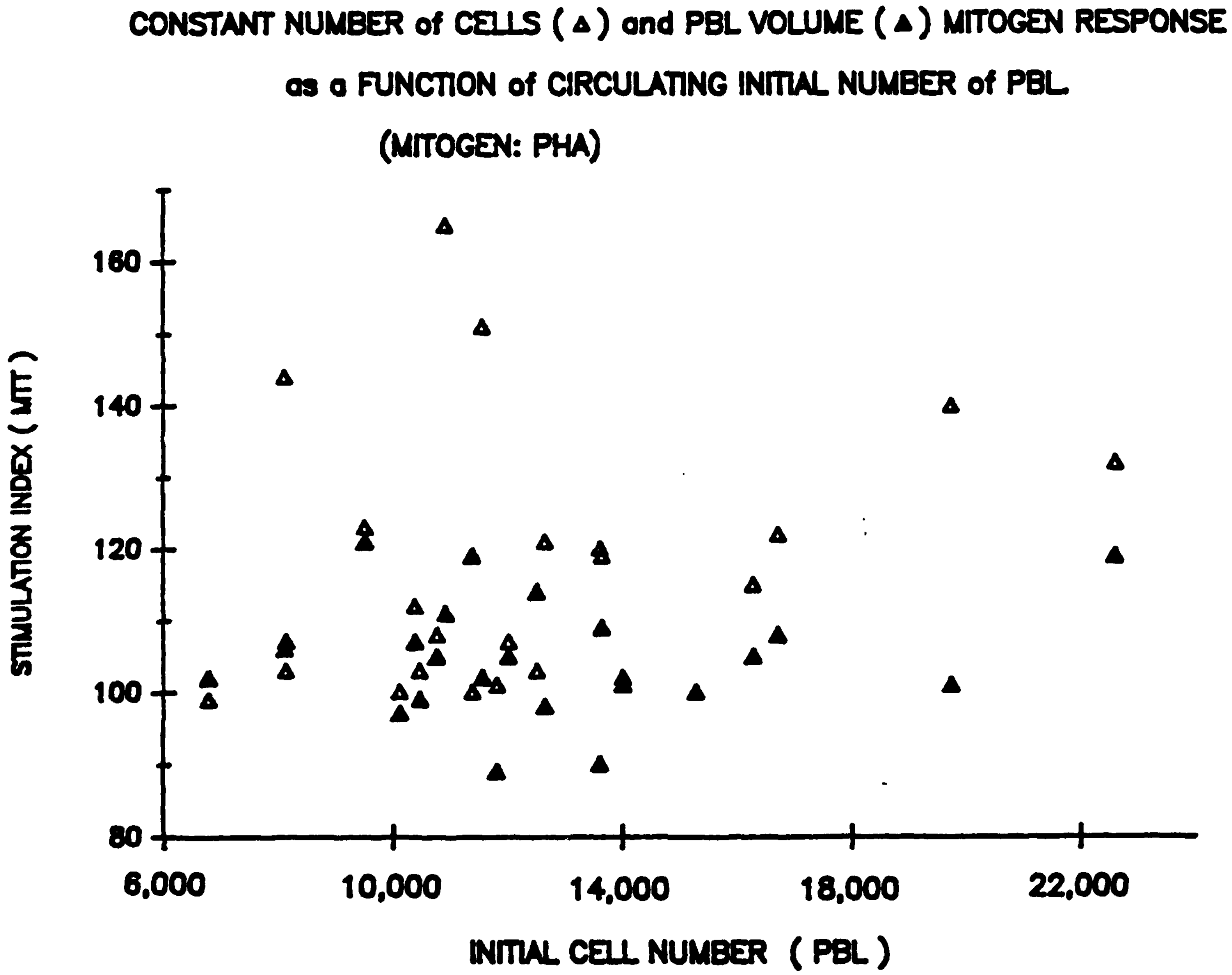


FIG. 21: COMPARISON OF METHODS OF EVALUATING PHA REACTIVITY AND NUMBER OF CIRCULATING PBL

E. Summary of Results.

1. Using PBL blast responses to the mitogens concanavalin A (ConA) and phytohemagglutinin (PHA) as indicants of immune function, the project has demonstrated that social behavior manipulations designed to induce social stress in our monkey groups can produce changes in mitogen stimulation indexes (SI's). Patterns of response to ConA were different from those to PHA stimulation, indicating that the two mitogens were stimulating different populations of cells. The response to ConA stimuation was more closely associated with social manipulations and social behavior than PHA. Generally, social manipulations suppressed SI's and this was followed by a rebound effect. In some instances, strangers introduced into an established group had elevated SI's on the day of introduction.

2. The use of a physical stressor - electric footshock - produced suppression of SI's to both ConA and PHA in the first shock experiment and, once again, the patterns of response were different for the two mitogens. A second footshock experiment was conducted to provide better information about changes during and after repeated stress. This experiment, in contrast to the first shock study, provided for the testing of social behavior on the days shock was administered. The initial number of PBL's in 1 ul of fresh whole blood was lower in each monkey at some point during the 13 days of shock and this was followed by an adaptation period. Following termination of shock, there was a rebound effect after shock was terminated in which the count overshot the baseline values before returning to a level slightly below baseline. ConA stimulation produced low SI's on the first day of shock and were highest four days after shock was terminated. Variability was greatest on the last shock day and four days post shock, suggesting that repeated shock experiences magnified differences in individual response patterns. Because of thh large variances, the overall statistical analysis was not significant. In contrast to the first shock study which used the thymidine assay, SI's to PHA, using the MTT assay, did not change consistently.

3. The social data obtained during the second shock study revealed two findings of potential importance. Reversals in rank which occurred during the experiment were associated with increased ConA SI's in winners and decreased SI's in losers. There was an increase in both aggressive and affiliative behaviors over preshock levels during the first five days of shock and then affiliative behavior scores dropped to very low levels during the last eight shock days. The increasing aggressive and affiliative responses were associated with rising SI's to ConA stimulation (shock day 5), depressed social scores with falling SI's (shock days 9 and 13), and increasing affiliative scores with rising SI's after shock termination

(postshock day 4). There appears to be an interaction between the administration of the physical stressor and alterations in social behavior - particularly affiliative behavior - which is reflected in consistent changes in ConA SI's.

4. Attempts to correlate prolactin (PRL) levels with SI's to mitogen stimulation were largely unsuccessful. PRL values tended to be very low during both physical and social stress. The low readings might be due to depletion, active inhibition, or habituation to a particular stressor. Further study of these three possibilities will be required to understand the mechanisms involved so that we can proceded with study of relationships between PRL and immune function. Because of the drop in PRL with chronic (social) or repeated (footshock) stress, we interpret the action of PRL to be a trigger (not a sustaining) mechanism in immunofacilitation. In any event, it will be necessary to measure PRL earlier in social stress situations since, as a pituitary hormone, it probably peaks within 15-30 min of the administration of a stressor. As expected, serum cortisol values generally increased during agonistic interactions and social manipulations. The levels decreased across days of repeated footshock, suggesting that habituation to shock was occurring. ACTH values also increased, but the data were more variable than for cortisol. ACTH did not contribute any additional information about the nature of the stress beyond that obtainable from cortisol. Its potential usefulness in future work lies in its role as an immunomodulator rather than as a simple indicant of stress. Responses on a multiple RI RI operant schedule proved to be very sensitive to the social manipulations. The response suppression that occurred following the introduction of strangers to the group suggests that this, or a similar, performance measure could be very useful as either a supplement or an alternative to the hormonal criteria for stress.

5. Because of initial difficulties with the MTT colorimetric assay, the first studies used the ^3H thymidine assay for the mitogen stimulation work. After several false starts we adapted the MTT assay to use with our monkeys and were able to use it during the latter part of the year. We then made an effort to quantitate the differences between the two methods using aliquots from the same well of the tissue culture plates. We found a high correlation (r = +.96) between cell number and MTT optical density in unstimulated PBLs, but the correlation decreased as incubation time was increased following the addition of a mitogen. The optimal MTT stimulation index (SI) occurred after 48 hours of incubation. Although the optimal uptake of ^3H thymidine occurred after 96 hours of incubation (24 hr pulse with the ^3H label), the MTT assay demonstrated that MTT dye sensitive cells were rapidly dying. (see Figure 1). Microscopic examination showed that the predominant cell type at 96 hr had a different morphology and

only a small percentage of the cells took up the MTT dye.

A marked differences in individual monkey's PBL responses to mitogen stimuation was observed in both assays. These differences were observed even when constant initial cell numbers were used. Although there was a tendency for high MTT SIs to be accompanied by lower SIs for ^{3}H thymidine in individual monkeys, the effect was not statistically significant within the group of six animals investigated. We believe the low thymidine SI results from a deficiency of a growth factor of an unknown nature, since mitogen responsiveness could be restored by adding 5% PBL from another monkey.

The PBL in our monkey species is very responsive to Con A and PHA stimulation, but it is a very fragile lymphocyte to culture. Although we have tried several differeht incubation mediums and growth factors, we have not been successful in obtaining satisfactory mitogen responses from cultures of our monkey's lymphocytes after separating them from whole blood.

We had proposed to do IL-2 assays in the purified system, but were discouraged from doing so by reports from another laboratory which had been unsuccessful in inducing any changes in IL-2 in macaques through social manipulations. In consequence, we did not attempt any IL-2 assays during the past year; however, because responses to mitogen stimulation are such a crude index of immune system function, an expanded battery of assays has been incorporated into the proposal for continuing the present project. This includes an examination of IL-2 production following mitogen, antigen, and anti-T3 stimulation to document lymphocyte functional status. (See the next section of the report for details.)

6. Based on the data which indicated that social manipulations do alter measures of immune system function, a plan for continuation of this work has been developed and submitted. The proposal for the future work is descibed briefly in the next section of the report.

Future Work

This section provides a summary of the research projected over the next two years. The first year of the project has been devoted primarily to demonstrating that social variables have an effect on indices of immune system function in our monkeys, to establishing a laboratory for conducting assays used to study immune function and validating some basic assay procedures, and to evaluating behavioral testing methods for use in inducing social stress and coping behavior. The proposal for a two extension of the project would focus on: 1.) Data collection and analyses involving specific social behavioral

factors and situations that may enhance or suppress immunocompetence in normally healthy animals; 2.) expanding the list of measures of immune response potential to increase the likelihood of detecting relationships between such indicants and behavior; 3.) adding a procedure to assess socially induced changes in an ongoing response system which will be done by examining serum antibody responses to keyhole limpet hemocyanin (KLH) in immunized monkeys, and 4) utilizing the behavioral, hormonal, and immune function data to develop a model of the control functions which mediate the interactions between molar behavior on the one hand and neuroendocrine and immune responses on the other. Such a model would be useful in generating hypotheses about the functions of the immune/neural/endocrine control system in responses to stress by healthy individuals.

The paragraphs in this section present a brief summary of the behavioral experiments projected over the next two years. Depending upon the results of the initial experiments, modifications to the schedule outlined below may be required. We will be evaluating several procedures for manipulating social behavior and social stress. Those that are most effective in producing changes in immune system function will be retained for use in the elucidation of cellular and physiological mechanisms linking social, endocrine, and immune functions. Others will be dropped from the schedule as soon as it is determined that they do not work at all, or that they are less effective than other available procedures. It may be necessary to increase or decrease the intensity of the social stressors by altering the experimental manipulations of group membership and structure. The use of a nonsocial, physical stressor to validate the assays employed in assessing the effects of social variables on immune function will be kept to a minimum. However, we need to do two followup studies on neuroendocrine responses to footshock.

The projected experiments are categorized in terms of the manipulations to be made; within each category, experiments are listed in probable chronological order. Experiments in two or more categories may be carried out simultaneously:

<u>Removal from the Social Group</u>:

Experiment 1. Peer separation and reintroduction. The purpose is to gather information on the effects of peer separation on immune function in healthy, young adults. Four young adult males, ages 5-8 years, and four nonpregnant, young adult females, ages 4-6 years will be removed from one troop and housed individually. Two males and two females will be confined to their cages during the separation period. The remaining four monkeys will be be given daily 40-60 min individual training sessions on an operant schedule in the large indoor cage to

control for the possible effects of the restriction of space and activity produced by individual caging. (Incidentally, this control also addresses, in experimental terms, the issue of the effects of activity and exercise on the well being of laboratory housed primates.) Blood samples for assays of immune function will be obtained at weekly intervals for four weeks prior to removal from the troop, twice a week during separation, and twice a week following reintroduction. Separation will last a minimum or eight weeks, or until the indicants of immune function have stabilized. Following reintroduction, they will be monitored until they are behaviorally reintegrated into the social group and their immune system indicants have stabilized. Estimated duration of the study is 16 weeks.

Experiment 2. The purpose is to begin the development of an experimental model for bereavement. Immune system functions in six pregnant and three nonpregnant females will be monitored during the last month of pregnancy. One week following parturition, the infants will be removed from three females and hand reared in the laboratory. Changes in immune function in the "bereaved" mothers will be compared with those of the three females allowed to rear their infants normally and the three nonmothers. The infants that are hand reared will be group housed as soon as they are old enough and returned to their troops as soon as they are able to feed themselves. Duration of the study is expected to be 12-16 weeks. It is anticipated that the study will be conducted during the primary breeding season of the second year of the two year renewal period. If the results are promising, an extension of the project will be sought for further development and application of the model.

Introduction and Replacement:

Experiment 1. The purpose is to study the effects of social stress on immune function using removals and replacement of high ranking animals to induce moderate levels of stress. During the first year of the project, the primary method of inducing social stress was to introduce strangers to the social groups. This was clearly stressful to the monkey being introduced to the group, but the effect on the other members of the group depended, in large part, on the behavior of the stranger. We will continue to employ the introduction of strangers in our studies (See Experiment 3 in this section), but will evaluate a removal/replacement procedure to determine its effects on immune system indicants. Some of our earlier work had shown that removing a high ranking male from a group for two weeks or more produces a significant increase in agonistic interactions throughout the troop on the days following the reintroduction of the male. In a stable social group, most of these agonistic encounters do not involve contact aggression and there is a low potential for injury with

this procedure. Nevertheless, elevations in plasma glucocorticoids indicate that social stress is present. Removal and replacement of low ranking animals produces little or no change in agonistic social behavior or hormonal indicants of stress and is used as a control. To obtain sufficient data for statistical evaluation of immune system changes, we expect that between 6 - 10 high rank/low rank removal replacement procedures will be used during the project. Two or three manipulations would be done with each of the three groups living in the outdoor compounds. Each manipulation involves the collection of two weeks of premoval baseline assay data, a two week removal of the stimulus animals, and a minimum 4 week postintroduction phase. Measures of immune function will be monitored throughout in representative adult males and adult females, as well as juveniles of both sexes. During each manipulation, immune function information will be obtained from 6 - 14 animals depending on the particular group involved. Time involved in the conduct of the experiments will be shortened when we can use the data from the latter part of a postremoval period as the preremoval data for a second manipulation, etc.

Experiment 1a. Clique removal/replacement. This is a pilot experiment designed to evaluate the effects of removing and replacing sets of males instead of selecting individuals on the basis of their social status as in experiment 1. It is designed to take advantage of data obtained from one of the groups during the summer and fall of 1988 which indicated the presence of two opposing multianimal alliances ("cliques") within the adult male hierarchy of the troop. Members of each clique (one containing 3 and the other 4 monkeys) actively enlist the support of its members against members of the other clique and show higher frequencies of affiliative behaviors within the cliques than with other animals. In a preliminary study, the effects of removing and replacing one of the cliques on the behavior of the remainder of the troop will be evaluated using a schedule similar to that described for experiment 1. If successful, the removal and replacement of sets of animals, selected on the basis of their compatability/incompatibility, may be a viable and economical alternative to the high rank/low rank removal and replacement procedure.

Experiment 2. Matriarchy manipulations. Because the hierarchy of matriarchies is so important to the social structure and stability of the group, an experiment will be conducted to determine the effects of removing and replacing matriarchs in the two breeding troops. Matriarchs will be removed and replaced following a schedule similar to that of experiment 1. Changes in social behavior and immune function will be monitored in her matriarchy and in selected animals from other matriarchies. As many as four such manipulations are contemplated over the two years; however, the effectiveness of the procedure will be evaluated after each study and a

determination made as to its utility for further research. Depending on the size of the matriarchy, 10-14 monkeys will be monitored in each study.

Experiment 3. Introduction of strangers to existing groups. This procedure is stressful to the animal being introduced and allows the study of both the effects of acute defeat on immune system function and the recovery processes which should parallel their acceptance by the other members of the group. The purpose of this experiment is to look at individual differences in changes in immune response as a function of the way in which stranger animals cope with the new social environment. Monkeys which behave in ways which result in their integration into the social structure of their groups relatively quickly would be predicted to show greater immunocompetence than those that do not. Integration into the group is defined in terms of reductions in aggression and increases in affiliative behaviors such as allogrooming. In order to bring some new genetic stock into the present colony, we plan to acquire six males and six females over the two year period. We will take advantage of the introduction of these 12 animals into the breeding program by using them as subjects for this experiment. Should further use of this procedure be necessary in studies of coping behavior, exchanges of animals between existing groups, as done during the first year, would be employed.

Physical Stressors

During the first year a physical stressor, electric footshock, has been used in validating the mitogen assays so that we could get some idea of the range and nature of the responses we might expect to see in the social stress studies. A failure to detect a change in an indicant of immune function could be due to either a failure of the assay or the failure of the experimental conditions to produce a real change. Therefore it is necessary, from time to time, to cross validate the assay procedures used in the social studies with a stressor known to have an effect on the measure involved. In addition, one of the purposes of the project is to relate levels of serum prolactin (PRL) to changes in immune system activity induced by social stress. As we will be involved in measuring cytokine and antibody activity as well as lymphocyte proliferation during the coming year, additional use of the footshock stressor will be made in validating these assays. Also the data from the first year showed that PRL values tended to be lower than expected following both repeated footshock and chronic social stress. PRL secretion is known to habituate across days in stressed rats, but nothing is known about the pattern of the reduction in our monkeys. Similarly, nothing is known about the course of PRL secretion during an acute stress experience. Answers to both questions will be determined in two parametric

experiments and the data will also be used in interpreting some of the mitogen/PRL results obtained from the footshock and social behavior experiments done in the first year:

Experiment 1. The purpose is to determine the pattern of PRL secretion in response to acute footshock. This will also help us select the optimal window during which blood samples in the social experiments should be taken and we need to determine the maximum time allowable between removing and animal from a social group and obtaining a blood sample for PRL assay. Six monkeys that have never been exposed to shock will serve as subjects. They will be given six weekly 90 min sessions of footshock (1.8 ma delivered on a variable time (VT) 90 sec schedule; shock duration will be 3 sec). Blood samples will be obtained at 0, 10, 30, 60, 90, 120, and 150 min after the beginning of a session. Two 1 ml blood samples will be taken from each animal each week, one just prior to shock, and the other at one of the six times listed above. For the 10, 30, and 60 min samples, the animals will be removed from the chambers and then returned for the balance of the session; the 90, 120 and 150 min draws will be made after the session is concluded. The order of sampling will be counterbalanced across monkeys and each monkey will receive shock in a different chamber each week.

Experiment 2. Six monkeys that have never been exposed to footshock will be used to examine habituation of the PRL stress response across days. Ten consecutive days of 90 min sessions in the same chamber will be used with the same parameters as in experiment 1. Sampling (1.5 ml) times during shock will be selected on the basis of the data from experiment 1; if necessary, additional postshock sampling times will be used to determine the time needed for a return to baseline PRL levels in the repeated stress situation. This study will also be used in validating the new immune system assays.

Stress and Performance:

Work during the first year of the project has shown that performance on a multiple random interval (MULT RI 1-min RI 1-min) operant reinforcemnt schedule is very sensitive to social stress. We will continue to use this behavioral indicant of stress with our all male troop of six adults as a supplement to the hormonal indicants. The monkeys are already trained and we will simply monitor performance changes during the social group manipulations with this group.

We have six adult males that are housed in individual cages in the laboratory except when they are put together for social behavior tests in a large indoor observation cage. These are used to study competitive and cooperative behavior in a situation which requires them to work for food reward in a

social group situaton. An experiment from another laboratory with a different species of macaque has shown that immune system indicants can be altered when the monkeys are forced into social contact when feeding. In our tests, the monkeys have been trained to two use operant panels in the indoor social test cage to obtain food delivered on various reinforcement schedules. This provides a sophisticated way of generating social interactions among the animals obtaining food. Competition and cooperation are induced and manipulated by altering the schedule requirements on each panel and by forcing the animals to use only one or the other panel. We will explore a number of protocols during the comming months in order to determine their utility for producing or alleviating social stress and affecting immune system functions. Animals will be tested in pairs and triads as well as in the full six member group and during the introduction of a stranger.

An operant panel has been installed out of doors in one of the compounds containing one of the breeding troops. The panel can be programmed by a built in computer to deliver a variety of reinforcement schedules. We plan to extend the work described in the previous paragraph to a large, intact social group. This will increase the potential for studying behavior in a more complex social situation than is provided by the indoor social testing situation. The long range objective of this procedure is the development of a "closed economy" social environment such that the members of one of our monkey troops will be required to earn their daily food rations. A fairly extensive pilot project is proposed for the next two years to determine the practicality of installing a closed economy in a social group. If it works, it would be a powerful tool for investigating coping behavior as a function of social variables and could be used in the study of social stress and immune function.

REFERENCES

Ader, R., Grota, L. J. and Cohen, N. Conditioning phenomena and immune function.*Ann. NY Acad. Sci.* 1987, 496, 532-544.

Angst, W. basic data and concepts on the social organization of *Macaca fascicularis.* In *Primate Behavior.* (Rosenblum, L., ed.) New York: Academic Press, 1975.

Arora, P. K., Hanna, E. E., Paul, S. M. and Skolnick, P. Suppression of immune response by BDZ receptor reverse agonist. *J. Neuroimmunology*, 1987, 15, 1-9.

Berczi, I. *Pituitary Function and Immunity.* Boca Raton, Florida: CRC Press, 1986, 347pp.

Bernton, E. W., Hartmann, D., Gilbreath, M., Holaday, J. and Meltzer, M. S. Inhibition of macrophage in vivo activation by pharmacologic blockade of prolactin release. In *Leukocytes and Host Defense.* (Oppenheim, J. J. and Jacobs, D. M., eds.). New York: Alan Liss, 1986, 213-219.

Besedovsky, H., delRay, A., and Sorkin, E. Regulatory immune-neuroendocrine feedback signals. In: *Pituitary Function and Immunity* (I. Berczi, ed.), CRC Press, Boca Raton, FL, 1986, 241-249.

Besedovsky, H., delRay, A. and Sorkin, E. Immunological neuroendocrine feedback circuits. In: *Neural Modulation of Immunity* (R. Guillemin, M. Cohen and T. Melnechuk, eds.), Raven Press, NY, 1985, 165-177.

Buckley, A. R., Crowe, P. D. and Russell, D. A. Rapid activation of protein kinase C in isolated rat liver nuclei by prolactin, a known hepatic mitogen. *Proc. Soc. Natl. Acad. Sci.*, 1988, 85, 8649-8653

Buckley, A. R., Montgomery, D. W., Kibler, R., Putnam, C. W., Zukoski, C. F., Gout, P. W., Beer, C. T., and Russell, D. H. Prolactin stimulation of ornithine decarboxylase and mitogenesis in Nb2 node lymphoma cells. *Immunopharmacology.*, 1986, 12, 37-51.

Bunnell, B. N. Performance correlates of social behavior and organization in non-human primates. Final Technical Report, Contract No. DADA17-73-C-3007, USAMRDC, 1982.

Bunnell, B. N. and Iturrian, W. B. The effects of CW-related chemicals on social behavior and performance. *Annual Report Number 3/Final Report on USAMRDC Contract No. DAMD17-83-C-3260*, 1988.

Bunnell, B. N., Meyerhoff, J. L. and Kant, G. J. Psychological stress increases pituitary cyclic AMP. *Pharmacol. Biochem. Behav.* 1988, 29, 151-155.

Cooper, E. L. *Stress, Immunity, and Aging*. New York: Marcel Dekker, 1984.

Cunningham, A. J. Mind, body, and immune response. In: *Psychoneuroimmunology* (R. Ader, ed.), Academic Press, NY, 1981, 609-617.

Friedman, S. B., Glasgow, L. A. and Ader, R. Psychological factors modifying host resistance to experimental infections. *Ann. N. Y. Acad. Sci.*, 1969, 164, 381-392.

Glaser, R.,, Rice, J., Sheridan, J., Fertel, R., Stout, J., Speicher, C., Pinsky, D., Kotur, M., Post, A., Beck, M. and Kiecolt-Glaser, J. Stress-related immune suppression: Health implications. *Brain, Behavior, and Immunity*, 1987, 1, 7-20.

Gross, W. B. Effect of social stress on occurrence of Marek's disease in chickens. *Am. J. Vet. Res.*, 1972, 33, 2275-2279.

Guillemin, R., Cohen, M. and Melnechuk, T. *Neural Modulation of Immunity*. Raven Press, NY, 1985, 258 pp.

Hall, N. R. and Goldstein, A. L. Neurotransmitters and host defense. In: *Neural Modulation of Immunity* (R. Guillemin, M. Cohen and T. Melnechuk, eds.). New york: Raven Press, 1985, 143-156.

Hall, N. E., McGillis, J. P., Spangelo, B. L., Healy, D. L., Chrousos, G. P., Schulte, H. M., and Goldstein, A. L. Thymic hormone effects on the brain and neuroendocrine circuits. In: *Neural Modulaton of Immunity*, (R. Guillemin, M. Cohen and T. Melnechuk, eds.). New York: Raven Press, 1985, 179-193.

Jancovic', B. D. From immunoneurology to immunopsychiatry: Neuromodulating activity of antibrain antibodies. *Int. Rev. Neurobiol.*, 1985, 26, 249-314.

Jancovic', B. D. and Spector, N. H. Effects on the immune system of lesioning and stimulation of the nervous system: Neuroimmunomodulation. In: *Enkephalins and Endorphins: Stress and the Immune System* (N. P. Plotnikoff, R. E. Faith, A. J. Murgo and R. A. Good, eds.). New York: Plenum Press, 1986, 189-220.

Jancovic', B. D. and Maric', D. Enkephalins and autoimmunity: Differential effect of methionine-enkephalin on experimental allergic encephalomyelitis in Wistar and Lewis rats. *J. Neurosci. Res.*, 1987, 88-94.

Jancovic',B. D., Markovic', B. M. and Spector, N. H. (Eds.) *Neuroimmune Interactions: Proc. 2nd Int. Workshop on Neuroimmunomodulation. N.Y. Acad. Sci.*,1987, 496, 756pp.

Kant, G. J., Meyerhoff, J. L., Bunnell, B. N. and Lenox, R. H. Cyclic AMP and cyclic GMP response to stress in brain and pituitary: Stress elevates pituitary cyclic AMP. *Pharmacol. Biochem. Behav.* 1982, 17, 1067-1072.

Kant, G. J., Bunnell, B. N., Mougey, E. H., Pennington, L. L. and Meyerhoff, J. L. Effects of repeated stress on pituitary cyclic AMP, prolactin, corticosterone, and growth hormone in male rats. *Pharmacol. Biochem. Behav.* 1983, 18, 967-971.

Kummer, H. *Primate societies: Group techniques of ecological adaptation.* Arlington Heights, IL: AHM Publishing Corp., 1971.

Laird, H. E., Duerson, K., Buckley, A. R., Montgomery, D. W. and Russell, D. H. Peripheral benzodiazepine (BDZ) receptor enhances prolactin dependent mitogenesis in NB2 node lymphoma cells. *Fed. Proc.*, 1987, 46, 542.

Laudenslager, M. L., Held, P. and Boccia, M. Dominance and immunity in nonhuman primates: Some pilot observations. *Soc. Neurosci. Abstr.*, 1988, 14, 1281.

Laudenslager, M. L. Reite, M. L. and Harbeck, R. J. Suppressed immune response in infant monkeys associated with infant separation. *Behav. Neural. Biol.*, 1982, 36, 40-48.

Locke, S. E. and Hornig-Rohan, M. Mind and Immunity: Behavioral Immunology (1976-1982). Auburndale, MA: Elliot Press, 1983.

Locke, S., Ader, R., Besedovsky, H., Hall, N., Solomon, G. and Strom, T. (eds.), *Foundations of Psychoneuroimmunology.* New York: Aldine, 1985.

Lysle, D. T., Cunnick, J. E., Fowler, H. and Rabin, B. S. Pavlovian conditioning of shock-induced suppression of lymphocyte reactivity: Acquisition, extinction, and preexposure effects. *Life Sciences*, 1988, 42, 2185-2194.

MacLeod, R. M., Scapagnini, U., and Thorner, M. O. (eds.) *Prolactin: Basic and clinical correlates. Fidia Res. Series Vol. 1.* New York: Springer Verlag, 1985

Monjan, A. A. Effects of acute annd chronic stress upon lymphocyte blastogenesis in mice nd humans. In: *Stress, Immunity and Aging* (E. L. Cooper, ed.). New York: Marcel Dekker, 1984, 81-108.

Monjan, A. A. Stress and immunologic competence studies in animals. In: *Psychoneuroimmunology* (R. Ader, ed.). New York: Academic Press, 1981, 185-227.

Mossmann, T. Rapid colorimetric assay for cellular growth and survival: Applications to proliferation and cytotoxicity studies. *J. Immunology*, 1983, 65, 55-63.

Murgo, A. J. Faith, R. E. and Plotnikoff, N. P. Enkephalins: Mediators of stress induced immunomodulation. In N. P. Plotnikoff, et al, Eds.: *Enkephalins and Endorphins: Stress and the Immune System*. New York: Plenum, 1986, 221-240.

Nagy, E. and Berczi, I. Prolactin and other lactogenic hormones. In I. Berczi, Ed.: *Pituitary Function and Immunity*. Boca Raton, FL: CRC Press, 1986, 161-183.

Perez-Polo, J. R., Bulloch, K., Angeletti, R. H., Hashhim, G. A. and deVellis, J. (Eds.) *Neuroimmunomodulation*. New York: A. R. Liss, 1987.

Pericic', D., Manev, H., Boranic', M., Poljak-Blazi, M. and Lakic', N. Effect of diazepam on brain neurotransmitters, plasma corticosterone, and the immune system of stressed rats. *Ann. NY Acad. Sci.*, 1987, 496, 450-458.

Plaut, S. M. and Friedman, S. B. Psychosocial factors in infectious disease. In: *Psychoneuroimmunology* (R. Ader, ed.). New York: Academic Press, 1981, 3-29.

Plaut, S. M., Friedman, S. B. and Grota, L. J. *Plasmodium berghei*: Resistance to infection an group and individually housed mice. *Exp. Parasitol.*, 1971, 29, 47-52.

Plotnikoff, N. P., Faith, R. E., Murgo, A. J. and Good, R. A. *Enkephalins and Endorphins: Stress and the Immune System*. New York: Plenum Press, 1986.

Rabin, B. S., Lyte, M., Epstein, L. H. and Caggiula, A. R. Alteration of immune competency by number of mice housed per cage. *Ann. N.Y. Acad. Sci.*, 1987, 496, 492-500.

Reite, M., Harbeck, R. and Hoffman, A. Altered cellular immune responses following peer separation. *Life Sci.*, 1981, 1133-1135.

Riley, V. Psychoneuroendocrine influences on immunocompetence and neoplasia. *Science*, 1981, 212, 1100-1109.

Russell, D. H., Kibler, R., Matrisian, L., Larson, D. F., Poulos, B. and Magun, B. E. Prolactin receptors on rat lymphoid tissues and on human T and B lymphocytes: Antagonism of prolactin binding by cyclosporine. In: *Prolactin: Basic and Clinical Correlates* (R. M. MacLeod et al, eds.). New York: Springer-Verlag, 1985, 375-384.

Russell, D. H., Matrisian, L., Kibler, R., Larson, D. F., Poulos, B. and Magun, B. E. Prolactin receptors on human lymphocytes and their modulation by cyclosporine. *Biochem. Biophys. Res. Comm.*, 1984, 121, 899-906.

Schindler, B. A. Stress, affective disorders and immune function. *Med. Clin. N. Amer.*,1985, 69, 585-597.

Schleifer, S. J., Keller, S. E., Camerino, M., Thornton, J. C. and Stein, M. Suppression of lymphocyte stimulation following bereavement. *J. A. M. A.*, 1983, 250, 374-377.

Smith, E. M. and Blalock, J. E. A complete regulatory loop between the immune and neuroendocrine systems operates through common signal molecules (hormones) and receptors. In: *Enkephalins and Endorphins: Stress and the Immune System* (N. P. Plotnikoff, et al, eds.). New York: Plenum Press, 1986, 119-128.

Soloman, G. F. Psychoneuroimmunology: Interactions between central nervous system and immune system *J. Neurosci. Res.*, 1987, 18, 1-9.

Solomon, G. F. and Amkraut, A. A. Psychoneuroendocrinological effects on the immune response. *Ann. Rev. Microbiol.* 1981, 35, 155-184.

Solomon, G. F., Levine, S. and Kraft, J. K. Early experience and immunity. *Nature*, 1968, 220, 821-822.

Spangelo, B. L., Hall, N. R. and Goldstein, A. L. Evidence that prolactin is an immunomodulatory hormone. In: *Prolactin: Basic and Clinical Correlates.* (R. M. MacLeod, et al, eds.). New York: Springer-Verlag, 1985, 342-349.

Spector, N. H. Anatomical and physiological connections between the central nervous system and the immune systems. In: *Immunoregulation* (N. Fabris, E. Garaci, J. Hadden and N. A. Mitchison (eds). New York: Plenum Press, 1983, 231-258.

Spector, N. H. Old and new strategies in the conditioning of immune responses. *Ann. NY Acad. Sci.*, 1987, 496, 522-531.

Stein, M. Bereavement, depression, stress and immunity. In: *Neural Modulation of Immunity* (R. Guillemin, et al, eds.). New York: Raven Press, 1985, 29-44.

Steplewski, Z. and Vogel, W. H. Total leukocytes - T cells subpopulation and natural killer (NK) cell activity in rats exposed to restraint stress. *Life Sci.*, 1986, 38, 2418-2427.

Tecoma, E. S. and Huey, L. Y. Psychic distress and the immune response. *Life Sci.*, 1985, 36, 1799-1812.

Vessey, S. H. Effects of grouping on levels of circulating antibodies in mice. *Proc. Soc. Exp. Biol. Med.*, 1964, 115, 252-255.

Wechsler, B. Behavior of an alpha male in a captive grouup of crab-eating macaques (*Macaca fascicularis*). *Folia Primatologica*, 1986, 4, 91-97.

APPENDIX A

Sample Social Behavior Matrices
I-Troop, September 1988

Social Behavior in I-Troop: The following tables are the matrices derived from the analyses of 5 days of 40 min group scan social data recorded from I-Troop during the period 1 September 26 - 30, 1988. I-Troop was intact during this time - all 6 adult male monkeys were present during all observation periods. The matrices labeled SUBMISSIVE, AGGRESSIVE, AFFILIATIVE, and SEXUAL contain the behaviors listed in these categories in Table 2 on page 17 of the body of the report. The GROOMS matrix is for social grooming (allogrooming) and contains this behavior only. GROOMS is contained within the AFFILIATIVE matrix as well. In each matrix, the frequency with which each monkey directs a given class of behavior toward every other animal in the troop is read across the horizontal rows. The frequency with which each monkey receives each class of behavior is read down the vertical columns. Row, column, and matrix totals are at the right margin and the bottom of each matrix. The SUBMISSIVE matrix establishes the social rank hierarchy in terms of who submits to whom. The other matrices are constructed using this same order. Notice that the SUBMISSIVE matrix shows that 14 of the 15 possible dominance/submission relationships have been identified during the 5 observation periods. Only the relationship between Equal and Quotation was not directly observed.

SUBMISSIVE:

	YUK	HORAT	YAMAM	EQUAL	QUOTA	RHETO	TOTAL
YUK		0	0	0	0	0	0
HORATIO	1		0	0	0	0	1
YAMAMOTO	2	2		0	0	0	4
EQUAL	1	1	1		0	0	3
QUOTATION	10	2	1	0		0	13
RHETORIC	6	3	4	4	1		18
TOTAL	20	8	6	4	1	0	37

APPENDIX A (Continued)

AGGRESSIVE:

	YUK	HORAT	YAMAM	EQUAL	QUOTE	RHETO	TOTAL
YUK		0	0	0	0	2	2
HORATIO	0		0	0	0	0	0
YAMAMOTO	0	0		0	0	0	0
EQUAL	0	0	0		0	3	3
QUOTATION	0	0	0	0		0	0
RHETORIC	0	0	0	0	0		0
TOTAL	0	0	0	0	0	5	5

AFFILIATIVE:

	YUK	HORAT	YAMAM	EQUAL	QUOTA	RHETO	TOTAL
YUK		0	2	2	3	1	8
HORATIO	2		7	8	0	0	17
YAMAMOTO	5	2		13	4	0	24
EQUAL	2	6	13		16	0	37
QUOTATION	4	3	2	9		2	20
RHETORIC	0	1	0	0	0	0	1
TOTAL	13	12	24	32	23	3	107

Note the absence of aggressive behavior even though enough submissive behavior occurred to establish the social hierarchy. Even though Rhetoric had been in the troop for three months, his low affiliative behavior scores indicate he was not well integrated into the social structure of the group. Horatio, a member of the troop for just two months, had established affiliative bonds with the other members at this time.

APPENDIX A (Continued)

SEXUAL:

	YUK	HORAT	YAMAM	EQUAL	QUOTE	RHETO	TOTAL
YUK		0	0	1	0	0	1
HORATIO	0		0	2	0	0	2
YAMAMOTO	0	0		1	0	0	1
EQUAL	0	0	0		0	0	0
QUOTATION	0	0	0	0		0	0
RHETORIC	0	0	0	0	0		0
TOTAL	0	0	0	4	0	0	4

GROOMS:

	YUK	HORAT	YAMAM	EQUAL	QUOTA	RHETO	TOTAL
YUK		0	2	1	1	0	4
HORATIO	1		2	2	0	0	5
YAMAMOTO	2	1		8	1	0	12
EQUAL	1	3	4		8	0	16
QUOTATION	2	1	1	4		1	9
RHETORIC	0	0	0	0	0	0	0
TOTAL	6	5	9	15	10	1	46

APPENDIX B

Auxiliary Data Tables

ACTH Assays. The following table gives some of the ACTH assay results that were not incorporated into the body of the main report. The August NT-Troop samples were assayed separately from the September samples. The somewhat lower ACTH values in September probably reflect the fact that the September samples had been thawed and refrozen twice for the PRL and cortisol assays before being assayed for ACTH. The I-Troop assays of the July and August samples were performed in December after these samples had been assayed for PRL and cortisol much earlier. For purposes of comparison, we obtained fresher samples after the completeion of the project and assayed them for ACTH first. It will be seen that the values for the fresher samples that had not been previously thawed and refrozen were significantly larger than the old samples. The samples in the table were all taken while no social manipulations were being conducted with I-Troop

Serum ACTH levels in NT-Troop During Social Introductions August - September, 1 988. (Value in parenthesis indicates animal not in troop.)

ACTH (pg/ml)

Date:	8/22 Patek Intro	8/26 Group Intact	9/19 Ouezel Out	9/23 Rasputin Intro	9/27 Alien Intro	9/30 Group Intact
Animal:						
Patek	141.4	74.8	64.9	58.5	40.0	51.9
Allen	71.9	76.0	36.3	37.4	17.5	32.6
Barker	124.6	67.1	60.9	68.0	50.4	60.9
Hobbit	45.2	55.3	49.1	37.9	8.3	34.9
Kukla	102.7	86.2	50.1	54.6	31.6	46.3
Ouezel	156.4	130.4	(71.5)	(67.2)	(49.0)	58.6
Duster	Not Assayed		64.0	64.9	52.2	48.9
Achmed	Not Assayed		72.9	no sample	57.9	54.2
Rasputin	(--no samples--)		(63.6)	37.5	79.6	64.4
Alien	(-------no samples-------)			(61.9)	61.1	71.0
Dusty	73.6	103.2	62.8	57.8	55.6	65.2
Lilly	Not Assayed		54.2	52.7	39.2	42.7
Kilgore	131.9	147.5	79.1	74.9	66.7	60.9
Dahlia	Not Assayed		63.5	72.2	43.1	59.3

Appendix B (Continued)

Serum ACTH levels in I-Troop. (July-August samples assayed 12/14/88; December-January samples assayed 1/10/89.)

ACTH (pg/ml)

Date:	7/15	8/5	8/8	8/12	12/2	12/9	12/16	1/6
Animal								
Yamamoto	29.8	31.4	31.6	27.0	51.4	67.9	78.2	79.3
Yuk	27.0	33.4	--	39.8	72.3	67.9	80.9	94.5
Equal	--	31.5	39.6	44.6	54.8	58.5	61.3	74.9
Quotation	35.9	32.8	34.9	56.7	79.8	74.8	77.1	80.9
Rhetoric	38.3	24.7	29.7	29.1	(63.3)	(77.8)	(87.2)	(84.7)
Horatio	no sample	29.0	27.7	29.2	60.7	62.6	75.8	(62.9)

APPENDIX C

Personnel and Other Matters

1. The following people were employed on the project:

Name	Position	% Effort	Dates Employed
B. N. Bunnell	PI	25%	10/87- 1/89
W. B. Iturrian	Co-PI	10%	10/87- 1/89
M. A. Hebert	Grad Asst.	33%	10/87-12/88
J. L. Weed	Grad Asst.	33%	1/88- 9/88
R. C. Kyes	Grad Asst.	33%	7/88- 9/88
Z. Q. Li	Grad Asst.	33%	10/88- 1/89
M. Merlin	Student Asst.	25%	4/88-11/88
B. L. Krishniah	Lab Technician	100%	3/88- 4/88
H. Y. Liang	Lab Technician	100%	5/88- 1/89
T. Maddox	Caretaker	100%	10/87- 4/88
M. R. Longino	Caretaker	100%	5/88- 1/89

2. No graduate assistant received a degree during the time he ws employed on the project.

3. To date there have been no publications resulting from work done on this contract. As such appear, we will submit four copies of each to the Command.

4. Major items of equipment purchased during the period of the contract included an incubator, a low temperature cabinet, an ELISA microwell plate reader and a culture hood. The freezer has remained in the monkey laboratory to store serum and plasma samples, but we moved the incubator, ELISA reader and culture hood to Dr. Iturrian's laboratory in the Pharmacy building in order to lessen the chanc es of contamination of the cell cultures. By the end of the summer, this laboratory was set up and licensed to conduct both the MTT and the thymidine assays as well as the hormone assays.

5. A small version of the monkey restraint device, necessary for restraining ju veniles and females while blood samples are obtained, was built locally and use d in the NT-Troop social behavior studies. As noted in the body of the report, a second operant panel was installed in the C-Troop social cage and the outdoor operant testing apparatus was modified and reinstalled in the NT-Troop compound.

6. Dr. Iturrian attended the FASEB 1988 Summer Research Conference on Neuroimmu nomodulation at Copper Mountain, Colorado, June 26 - July 1, 1988. His expenses were paid by

the University of Georgia. To faciliate his supervising of the hormone assays being conducted in Dr. Martin's laboratory, Dr. Bunnell applied for and received a radioisotope license for work in that laboratory. This license also allowed the storage of RIA kits in the monkey laboratory, a procedure designed to reduce problems associated with the receipt and storage of these materials.

APPENDIX D

Executive Summary

This report describes the work conducted during a one year exploratory project involving the development of a nonhuman primate model for studying the effects of social stress on functions of the immune system. The specific technical objectives were:

1. To develop a nonhuman primate model for examining the effects of social stress on neuroendocrine modulation of immune function.

2. To use this model to examine the relationship between stress induced alterations in levels of the hormone prolactin (PRL) and cellular immune functions.

3. To develop experimental hypotheses about the ways in which social variables might affect the reciprocal relationships between the neuroendocrine and immmune systems involved in the modulation of an organism's response to stress.

Several procedures for recording and manipulating social behavior and inducing social stress in groups of cynomolgous monkeys (*M. fascicularis*) were employed. Social stress was defined in terms of activation of the hypothalamic-pituitary-adrenal axis as measured by serum levels of cortisol and ACTH. The indicants of immune system function were PBL blast responses to stimulation by the mitogens ConA and PHA. Electric footshock was used in two studies to define the hormone and mitogen responses to a physical stressor so these could be compared to the effects of the social stressors. Social behavior scores were also obtained in the second of the the two footshock studies. Correlations were sought between responses to mitogen stimulation, serum levels of PRL, and social behavior.

1. The results demonstrated that social behavior manipulations designed to induce social stress in our monkey groups can produce changes in mitogen stimulation indexes (SI's). Patterns of response to ConA were different from those to PHA stimulation, indicating that the two mitogens were stimulating different populations of cells. The response to ConA stimuation was more closely associated with social manipulations and social behavior than PHA. Generally, social manipulations suppressed SI's and this was followed by a rebound effect. In some instances, strangers

introduced into an established group had elevated SI's on the day of introduction.

2. Electric footshock produced suppression of SI's to both ConA and PHA in the first shock experiment and, once again, the patterns of response were different for the two mitogens. The second footshock experiment was conducted to provide better information about changes during and after repeated stress. The initial number of PBL's in 1 ul of fresh whole blood was lower in each monkey at some point during the 13 days of shock and this was followed by an adaptation period. Following termination of shock, there was a rebound effect in which the count overshot the baseline values before returning to a level slightly below baseline. ConA stimulation produced low SI's on the first day of shock and were highest four days after shock was terminated. Variability was greatest on the last shock day and four days post shock, suggesting that repeated shock experiences magnified differences in individual response patterns. Because of the large variances, the overall statistical analysis was not significant. In contrast to the first shock study which used the thymidine assay, SI's to PHA, using the MTT assay, did not change consistently.

3. The social data obtained during the second shock study revealed two findings of potential importance. Reversals in rank which occurred during the experiment were associated with increased ConA SI's in winners and decreased SI's in losers. There was an increase in both aggressive and affiliative behaviors over preshock levels during the first five days of shock and then affiliative behavior scores dropped to very low levels during the last eight shock days. The increasing aggressive and affiliative responses were associated with rising SI's to ConA stimulation (shock day 5), depressed social scores with falling SI's (shock days 9 and 13), and increasing affiliative scores with rising SI's after shock termination (postshock day 4). There appears to be an interaction between the administration of the physical stressor and alterations in social behavior - particularly affiliative behavior - which is reflected in consistent changes in ConA SI's.

4. Attempts to correlate prolactin (PRL) levels with SI's to mitogen stimulation were largely unsuccessful. PRL values tended to be very low during both physical and social stress. The low readings might be due to depletion, active inhibition, or habituation to a particular stressor. Further study of these three possibilities will be required to understand the mechanisms involved so that we can proceded with study of relationships between PRL and immune function. Because of the drop in PRL with chronic (social) or repeated (footshock) stress, we interpret the action of PRL to be a

trigger (not a sustaining) mechanism in immunofacilitation. In future work it will be necessary to measure PRL earlier in social stress situations since, as a pituitary hormone, it probably peaks within 15-30 min of the administration of a stressor.

5. Because of initial difficulties with the MTT colorimetric assay, the first studies used the ^{3}H thymidine assay for the mitogen stimulation work. After several false starts we adapted the MTT assay to use with our monkeys. We then tried to quantitate the differences between the two methods using aliquots from the same well of the tissue culture plates. We found a high correlation (r = +.96) between cell number and MTT optical density in unstimulated PBLs, but the correlation decreased as incubation time was increased following the addition of a mitogen. The optimal MTT stimulation index (SI) occurred after 48 hours of incubation. Although the optimal uptake of ^{3}H thymidine occurred after 96 hours of incubation (24 hr pulse with the ^{3}H label), the MTT assay demonstrated that MTT dye sensitive cells were rapidly dying. Microscopic examination showed that the predominant cell type at 96 hr had a different morphology and only a small percentage of the cells took up the MTT dye.

A marked differences in individual monkey's PBL responses to mitogen stimuation was observed in both assays and for constant initial cell number as well. Although there was a tendency for high MTT SIs to be accompanied by lower SIs for ^{3}H thymidine in individual monkeys, the effect was not statistically significant within the group of six animals investigated. The low thymidine SI probably results from a deficiency of a growth factor of unknown nature, since mitogen responsiveness could be restored by adding 10% PBL from another monkey.

The PBL in our monkey species is very responsive to Con A and PHA stimulation, but it is a very fragile lymphocyte to culture. Although we have tried several different incubation mediums and growth factors, we have not been successful in obtaining satisfactory mitogen responses from cultures of our monkey's lymphocytes after separating them from whole blood.

6. Based on the data which indicated that social manipulations do alter measures of immune system function, a plan for continuation of this work has been developed and submitted.